The Journal of Parasitology

Volume 34 FEBRUARY, 1948 Number 1

EXPANDING HORIZONS IN THE RECOGNITION OF A PHYLUM

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I. INTRODUCTION

The field of systematics has often been ridiculed, especially by biologists engaged in other aspects of science. Occasionally the writings of some systematists have merited this lack of respect but this fact provides neither reason nor excuse for belittling the entire field. The chief cause for contempt of systematics has been a lack of appreciation on the part of other biologists for the methods and objectives of taxonomic work. There has been a wide spread but fallacious belief that physical and chemical phenomena are objective realities while species and other taxonomic concepts are purely subjective creations of the human imagination. The apparent disregard for taxonomy has some times been voiced in the figure which rates the taxonomists as the "hod-carriers" of science. Those who hold such derogatory attitude toward the objectives of the taxonomist seem to have forgotten that the mortar carried by the hod-carrier is the stuff which gives stability to the wall. Without cooperation of hod carriers, the walls of biology would be but a loose pile of toppling bricks or stones, lacking pattern and orderliness. However, if the taxonomist is rated as but a menial laborer whose function is that of carrying the cohesive mortar for the edifice of biology, how much lower in the social scale of scientific castes must be the student of nomenclature who inspects the bricks for conformity and rejects those with flaws before they weaken the wall. All too often faulty material escapes first inspection and then part of the structure must be torn down so that the defective units may be replaced. To carry the figure a bit farther, we might say that the student of nomenclature is the apprentice to the hod-carrier since it is he who moistens the sand of personal endeavor and the lime of public opinion with the water of common sense and precedent to form the mortar for binding together the units constituting the structure of taxonomy.

Instead of attempting to organize a brief in defense of the fields and practices of taxonomy and nomenclature, this address will be devoted to tracing the growth of information regarding a single phylum, the Acanthocephala, or thorny-headed worms. In its history this group provides excellent material to illustrate the steps in the gradual expansion and evaluation of concepts in taxonomy. In the earliest days before the status of this group was understood, species now regarded as belonging within it were promiscuously assigned to various genera now recognized as representing several distinct phyla. By easy stages the knowledge of detailed

structure has replaced crude generalizations about the morphology of members of this group until the Acanthocephala now stand as a closely knit but highly diversified group which merits recognition as a phylum. Gradually this status has become established through the concerted efforts of numerous investigators who have contributed to an understanding of the varied aspects of the problem.

The philosophical view point of biologists in the past half century has been pretty thoroughly dominated by the vain attempt to reduce the content of the biological sciences to the material concepts which gripped the chemists and physicists in the stolid belief that the atom is the only ultimate reality. In the past generation biologists, psychologists, and philosophers have been straining their efforts in an endeavor to make living things fit into a universe dominated wholly by the concepts of atoms and their reactions. One of the greatest short-comings of this point of view has been the failure to realize that integration and organization are just as objective and as real as the individual atoms and their subdivisions and the energy which are distinctive of all matter. Throughout the Cosmos the presence of systems is omnipresent from the level of the structure of the atom to the architecture of the galaxies. The same sort of integration permeates our thinking whether we are dealing with the ultimate composition of genes in the single germ cell, with the organization of an individual animal, the integration of a society of individuals or evidences of taxonomic groupings and interrelationships.

Since before the time of Aristotle, philosophers have sensed the importance of orderliness and integration throughout the universe but no one has voiced this conviction with more eloquence than the English poet and philosopher Alexander Pope who more than two hundred years ago expressed it in the simple words, "Order is heaven's first law." Gradual realization of the importance of order and system have come to dominate all fields of scientific thinking. In no field has the effect been more marked than in the realm of taxonomy. Throughout the generations there have been profound changes in man's thinking as to the ultimate causes of the orderliness in nature but while these speculations are stimulating as philosophical problems they are marginal to the welfare of science itself.

From the time of Linnaeus down to the era dominated by Louis Agassiz (1857) the taxonomists fairly regularly regarded each species as an ultimate act of the creative power of God. It should be recalled that the concept of common origin as explanation of similar structures had no place in the thinking of taxonomists until after 1859 when Darwin's "Origin of Species" laid the scientific foundation for a belief in organic evolution as the background of phylogenetic relationships.

Added to the view point provided by the concepts of evolution, new material for the tracing of lines of common heritage has been furnished by the physiologists who have acquired an understanding of proteins and antigens to the point where specificity may be analyzed on a chemical basis. Embryologists and cyto-geneticists have likewise discovered many new evidences for tracing taxonomic and phylogenetic relationships. Unfortunately the complicated life histories of the Acanthocephala render genetical approach to these problems difficult and at present impossible. Interests in all of the newer fields of investigation have provided a much broader base upon which taxonomy and classification of the future may be built.

Except for a fairly early appreciation of habitat and distribution as important in limiting each species, all of the early descriptions of species were based upon strictly

morphological features. Interests in the new fields of investigation have pyramided into a greatly expanded understanding of taxonomy and classification. One tendency away from pure morphology in taxonomy was led in America by C. B. Davenport (1899 et seq.) in the attempt to reduce species concepts to mathematical formulae. N. A. Cobb with his nematode formula (1892) furnishes probably the best example of an attempt to reduce specific descriptions of helminths to a purely mathematical basis, where detailed observations on the morphology of a specimen were ultimately transposed to a series of measurements in the form of a purely mathematical formula although the numerals were actual measurements of morphological structures and were meaningless as a mathematical expression except as interpreted in terms of proportions of morphological features. In reality, the formidable Cobb formula is only short-hand notation for morphological observations, recorded in fixed sequence.

For many groups of the parasitic worms developmental stages offer evidences of speciation not clearly observable in the morphology of the adult worms and in quite a number of instances studies on the physiological relationships with the host provide evidences of either specific or varietal differentiation along physiological lines although the morphology of the forms may be indistinguishable.

In spite of these new avenues for the expression of mathematical and physiological differences, the majority of taxonomists continue to utilize morphological characters in the description of species and in the recognition of genera and supergeneric groupings. The direct integration and correlation of form and function is explanation enough for this procedure. While the average taxonomist does not include physiological, cytological and mathematical formulae in his descriptions, this omission does not mean that he disclaims validity of evidence from these fields. He usually regards these observations as accessory facts.

Occasionally the belief in what constitutes a species departs very widely from the morphological concept. As an example I know of no instance more perfect than the attempt of Wilhelmi (1940) to use antigen tests as a criterion for species. In his conclusions he stated (1940: 84):

"Species of helminths may be defined tentatively as a group of organisms the lipid-free antigen of which, when diluted to 1:4000 or more, yields a positive precipitin test within one hour with rabbit antiserum produced by injecting 40 mg of dry weight, lipid-free antigenic material and withdrawn ten to twelve days after the last of four intravenous injections administered every third day."

New evaluations of living things and their relations among themselves as expressed in classification and taxonomy, in heredity, in developmental processes, in physiological conditions, and in ecological relationships continue to appear. When taxonomy began to receive illumination from these varied new approaches it was no longer on the defensive for a place of respectability in the realm of science. It has developed new techniques and new objectives. At least potentially it has become elevated to the rank of an experimental science with advocates like Julian Huxley (1940) arguing in favor of "The New Systematics."

In giving consideration to any group of animals, before all of the advances offered by the new lines of evidence can be applied and evaluated in a system of taxonomy, the achievements thus far accomplished must be reviewed. For many years the writer has been especially interested in the numerous, intricate and confused problems of taxonomy and nomenclature in the Acanthocephala. Some of the horizons and background of history of this group will furnish the main topic for this address.

There is nothing distinctively peculiar in the history of taxonomy and nomenclature of the Acanthocephala. The members of this group have gone through their high as well as their low points in the processes of unfolding a knowledge of their classification very similar to the history of every other group of the worms. Several rather clearly marked epochs are recognizable in this history. While each of these periods has reasonably distinct time limits often they chronologically overlap one another and some run more or less continuously throughout, but with spurts of activity rather than gradual acceleration of rate of accomplishment marking the path of progress. For convenience in this paper, four epochs are recognized in the historical development of taxonomy and nomenclature of the Acanthocephala. The earliest of these horizons is the period of initial recognition of individuals, characterized by the first observations and fragmentary non-specific descriptions. The second era is recognizable as the time of significant discoveries, chiefly in morphology and ontogeny, marked by detailed studies of intimate external and internal structure and of the rough outline of the life histories of a few forms. The knowledge of morphology and ontogeny furnished the threshold for the third era when the recognition and evaluation of varying degrees of likeness and of difference resulted in the early attempts at tentative groupings of species into a limited number of genera and families. Throughout the entire history of the treatment of this group there has been continuous shifting of opinion as to the over-all relationships of the Acanthocephala to other groups of the parasitic worms. The fourth topic is the period of phylogenetic groupings and placement of the Acanthocephala within the general scheme of animal classification. For convenience of organization each of these periods will be discussed in turn even though the last topic has run continuously through the entire history of the group, and is not strictly a separate, isolated aspect of the problem.

II. THE INITIAL RECOGNITION OF INDIVIDUALS AND OF SPECIES

There are many of the groups of the animal kingdom which were better known to the ancient and classical world than were the thorny-headed worms. However, some of the very early microscopists described or figured parasites which are now recognized as belonging to the Acanthocephala. In the history of this material, varied editions of the works of the early naturalists are responsible for differences of opinion regarding the credit for being the one to record the first account of an acanthocephalan. Lühe (1904: 143) regarded Redi (1684) as the first author to offer a recognizable account of these worms. Meyer (1932: 4) has considered the Dutch microscopist von Leeuwenhoek (1695) as having given the earliest record in his description of two kinds of specimens which he found in an eel. It is now obvious that Lühe did not have access to the earlier edition of Leeuwenhoek (1695) available to Meyer and Meyer cited 1708 as date for Redi's observations while Lühe regarded the date for Redi as 1684. Mistaken dates are among the most frequently encountered causes for argument and misunderstanding in taxonomy and nomenclature.

Even in recent times, publishers are very lax in recording dates of publications. There are three top-ranking works on Acanthocephala for which the publishers are responsible for widespread misquotation of year of publication. I refer to the morphological monograph by J. E. Kaiser (1891–3), the significant historical treatment of nomenclature by M. Lühe (1904–5), and the comprehensive taxonomic and biological monograph by A. Meyer (1932–3). Each of these appeared in a publica-

tion which was issued in parts. After the separate sections were assembled as a complete volume or as a reprint, the publisher affixed the latest date on the cover and on the title page. Endless misunderstandings result from policies of this sort which are but little worse than the practice of putting a meaningless date on a serial publication to satisfy postal requirements, even though the actual date of distribution may be in a year entirely different from the recorded date of issue.

In the history of parasitology there is another type of error in dating publications which has been a serious obstacle to agreement on matters of priority and nomenclature. There was a time when some of the European periodicals accepted articles far beyond their ability to include them in current issues. As appeasement to the author such an article was often put into type and was then issued as an author's reprint under a date possibly a year or more before the appearance of the number of the periodical in which it ultimately appeared.

According to Meyer (1932: 4), von Leeuwenhoek presented a drawing of a proboscis which Meyer regarded as unmistakably that of *Acanthocephalus anguillae*. Lühe (1904: 145) was just as firm in the belief that the form observed by von Leeuwenhoek was *Echinorhynchus lucii*. The details of morphology upon which genera of Acanthocephala are recognized were not thoroughly understood for nearly two hundred years after the writings of the Dutch microscopist. This is an instance of the complete futility of any attempt at naming species and genera from the inadequate descriptions of early writers.

Naturally, formal binomial names were not applied by von Leeuwenhoek and other early observers of Acanthocephala but their descriptions and drawings leave no basis of doubt but that they were observing acanthocephalans. Names like Taenia, Lumbricus, Fasciola, Dracunculus and Ascarides were in relatively general usage by classical authorities as names for animal parasites before the dawn of any definite system of taxonomy. Many of the pre-Linnaean writers on medicine and on zoology used these names freely before the binomial system of naming had been invented. Often a species was so readily characterized by a single word indicating its host or some conspicuous feature about it that one or two words constituted a description. *Taenia canina* is a good example of such a terse, two-word description which often appeared at the opening of a new paragraph or as a center heading and then was set off in type different from that of the body of the text. This and several apparently sound binomial names were included in the work of Daniel Le Clerc (1715) long before Linnaeus had begun to formulate his system. Incidentally, Le Clerc, in many of his passages where seemingly "good" names were used, was quoting from still earlier writers. Were it not for the fact that the International Rules of Zoological Nomenclature fixed 1758, the date of publication of the Tenth Edition of Linnaeus's Systema Naturae, as the starting point of zoological nomenclature these headings would give no end of difficulty in establishing the origin of many of the binomial or binominal names among the parasitic worms. As the speaker has pointed out in another place (Van Cleave, 1936), a very great number of these classical names were later adopted by Linnaeus and although in their pre-Linnaean usage they have no claim to validity they furnish an interesting exhibit in the assembled evidences which seem to indicate that Linnaeus did not "invent" binomial nomenclature. That was a principle well understood and widely used for hundreds of species by men like John Ray and numerous others of his generation. In this connection it is in-

teresting to observe that although Linnaeus early adopted the general principle of binomial nomenclature he never consistently perfected it in his general writings until the tenth edition of the Systema Naturae appeared in 1758. In all earlier editions, many kinds of animals were characterized in relatively lengthy descriptions which passed in lieu of distinctive names cut to the binomial pattern. There was little understanding of fundamental structure and distinctive differences between the varied worm groups by pre-Linnaean writers and even by writers after Linnaeus began to perfect his system.

Except for one practically still-born generic name, no distinctive term was ever proposed to contain the species which are now recognized as thorny-headed worms until 1776, just two years before the death of Linnaeus.

No member of this group was ever granted recognition as a distinct specific or generic concept in any edition of the Systema Naturae prepared by Linnaeus or in any of the writings of his immediate contemporaries. In the 1758 edition of the Systema Naturae, it is apparent that Linnaeus at that time had never entertained any concept which can be recognized as the equivalent of any genus or other larger subdivision of the Acanthocephala. This statement carries the connotation that nomenclature for these forms is wholly post-Linnaean in its development.

The general unfamiliarity of Linnaeus with the parasitic worms seems to have been at least one reason which influenced Looss (1902: 736) to maintain that the system and the nomenclature of Rudolphi should be followed for the parasitic worms rather than that of Linnaeus. So far as the Acanthocephala are concerned, the works of Linnaeus are so lacking in information that the nominal establishment of the tenth edition of his monumental work as the starting point of nomenclature is practically meaningless. In fact, for several of the worm groups included in the heterogeneous "Vermes" of Linnaeus, the details of structure and development were apparently wholly unknown to him. Mere superficial external resemblances formed the only basis for his grouping together many of the forms for which morphological details and facts of life histories provided by studies in a later generation furnished the evidences for putting them asunder into widely separated groups representing several distinct phyla. Recognition of this line of advance in taxonomy is reflected in the view which is often expressed in the statement that the most significant modern advance in taxonomy of the invertebrates has centered around the resolution of the Vermes of Linnaeus into a multitude of discreet units representing all of the varied taxonomic levels up to and including phyla.

The thirteenth edition of the Systema Naturae, which Gmelin (1788–1793) edited after the death of Linnaeus in 1778, was the first of that series to treat of the species of Acanthocephala. In Volume 1, Part 6 (1790: 3024), Gmelin introduced Echinorhynchus as the first generic name for concepts of Acanthocephala to be given recognition in the Linnaean system. Some measure of the rate at which this fauna had been revealed in the generation following the death of Linnaeus is shown in the fact that Gmelin listed 48 species of the genus Echinorhynchus. He included Echinorhynchus in a wholly unresolved listing of roundworms, flatworms, and acanthocephalans under Linnaeus's "Vermes, Intestina" thereby revealing the fact that he regarded their habit of dwelling within other animals and lack of eyes ("Intra alia animalia degentia, oculis nullis") as the chief element of coherence in placing them together. This listing is entirely devoid of any basic facts of agreement in compara-

tive morphology, either external or internal. However, this lack of morphological evidence is not surprising when it is recalled that details of structure in these worms did not become soundly established or generally known for another full century beyond the time at which Gmelin was writing.

Linnaeus, in the twelfth edition of the Systema Naturae (1767: 1078), cited reference to *Fasciola barbata* as a parasite of the intestine of a cephalopod. Under this entry he listed *Taenia haeruca* Pallas, 1766 as a direct synonym. *Taenia haeruca* often has been accepted as a synonym of *Acanthocephalus ranae* but the *Fasciola barbata* is by common consent a cestode. Such confusion of synonymy involving not alone specific concepts but misallocation in wrong major taxonomic groups was not at all uncommon in the last half of the eighteenth century. According to Lühe (1905: 334), as early as 1819 Rudolphi regarded *Fasciola barbata* Linnaeus as a tetrarhynchid cestode. Among many recent authorities, Dollfus (1942: 71) has likewise considered *Fasciola barbata* as a cestode. It should be recalled that the groups Trematoda and Cestoda had not yet been given recognition in taxonomy at the time of Linnaeus. In fact the genera of parasitic worms prior to the 13th edition of the Systema Naturae were organized under two separate headings on features which gave no evidence of any appreciation of similarities and differences in fundamental structure. Fasciola, Ascaris and Lumbricus were in the heterogeneous assemblage which Linnaeus called the "Vermes, Intestina," while in the twelfth edition of the Systema Naturae (1767: 1323) Taenia was placed along with sponges, coelenterates and some protozoans in the "Vermes, Zoophyta." It is evident that Linnaeus regarded the repeated units of a cestode as similar to the parts of a colonial hydroid and was either unaware of or disregarded internal structures in his comparisons. Gmelin (1790) corrected this error when he incorporated Taenia and the other cestodes in the "Vermes, Intestina" although from his arrangement he apparently saw no particularly close relationship between the several genera of cestodes which he recognized.

Viewed in the light of present day recognition of groups, the confusion in concepts by Linnaeus (1767) went much farther than the failure to distinguish between Cestoda and Trematoda. Supreme confusion of natural groups here expressed was rather wide spread when formal taxonomy and nomenclature were young. Among the early writers who first attached formal names to thorny-headed worms, many of them were entirely innocent of any knowledge of the important morphological features upon which species could be recognized with certainty and unified groups of species could be established. They saw and understood only the superficial resemblances which existed between widely separated forms. In the absence of detailed information on internal structure and life histories, the superficial structures and appearances which accompany the varied adaptations to the parasitic habit permitted the uncritical observer to see more points of agreement than of diversity between an ascarid and an acanthocephalan or between a tapeworm and an acanthocephalan. It was this level of unawareness which permitted Pallas (1781) to call the familiar thorny-headed worm of the hog *Taenia hirudinacea.*

P. S. Pallas, O. F. Müller, J. A. E. Goeze, F. P. Schrank, C. A. Rudolphi, J. G. H. Zeder, J. G. Bremser, K. M. Diesing, J. F. Gmelin, and A. H. L. Westrumb, have been immortalized by the frequence with which their names appear as an integral part of a specific name in lists of Acanthocephala. The efforts of these early

workers laid a foundation upon which all later taxonomy of the group is based. The hundreds of kinds of Acanthocephala which they recognized at the specific level provided the substrate from which generic and family concepts were crystallized out by the workers of later generations.

Unfortunately the descriptions of all species of that era were so lacking in specific characters that there is rarely agreement among modern authorities as to the recognition of the species concerned. A truly fascinating aspect of speculation regarding the identity of the species described by many of the early writers is the fact that except in rare instances where the original specimens are extant or were available to competent later workers, the identity and synonymy of the inadequately described species can *never* be determined with certainty and but rarely with a reasonable degree of probability. Even the most honest, enthusiastic and capable investigator finds himself resorting to esoteric material to bolster the wholly unrecognizable descriptions of most of the early writers on this group. As one result of this situation, many of the species from European hosts that have been regarded as common have never been adequately described or diagnosed even to this date. Very often the features cited originally in specific descriptions of these common forms were shown to be generic in scope when identical features were later encountered in distinct species from other regions of the earth. The responsibility for proving that the characters have generic rather than specific value has fallen almost exclusively to the lot of workers in countries other than Europe. This neglect of specific details by early investigators finds the literature still very short of adequate specific diagnoses for many of the common European species of Acanthocephala.

As we view the unresolved confusion in instances such as that of *Taenia haeruca,* against the background of abundant, accurate details of morphology available to the present generation, the realization that minute specific details are lacking in the early works on taxonomy seems trivial when compared with the fact that these early workers were not even aware of the gross morphological distinctions between major groups such as the Trematoda, Cestoda, Nematoda and Acanthocephala.

Because so very many instances of presumed host specificity among the Acanthocephala have broken down when morphological features have been adequately studied, many workers have come to regard host specificity in this group of parasites as a myth. This attitude is in some measure attributable to the fact that many of the workers at the close of the 18th and beginning of the 19th Century gave specific names to specimens which they discovered in hosts for which Acanthocephala had not been reported previously. These specific names, derived most often from the name of the host with no information on the structure of the worm, have in large measure been relegated to oblivion as *nomina nuda* thus creating a bias against any recognition of host specificity. In recent years some fascinating evidences to the contrary have been unfolding. Particularly in the fishes, a number of instances have been found where speciation of the host has been directly accompanied by speciation of its parasites (Van Cleave, 1945 and 1947). There are on record a number of instances where closely related species of fish host have been found to harbor distinct species of Acanthocephala indicating that the same genetic and environmental factors which permit speciation in isolated populations of an originally uniform stock of fish indirectly induce parallel evolution and speciation in the parasites for which the fishes provide the environment.

In the Acanthocephala, broad individual variability in features that have been regarded as specific exists in all but a few genera. In consequence, sub-species or varieties have always been accorded a very unimportant and insignificant rôle in the taxonomy of this group. Among recent workers there have been very few attempts to recognize subspecies and most of these have been by investigators unfamiliar with specimens of the European species. Especially among early American workers there was a tendency to regard specimens found on this continent as varieties of the European species but for Acanthocephala not a single instance of this sort has been validated. Critical evaluation of attempts in this direction has resulted most frequently in extending the continuous range of variability of a well known species or in recognizing a new species not even remotely compatible with the species under which it was supposed to be subsumed. Over the wide, continuous geographical range of a series of hosts supporting a given species of acanthocephalan, the tendency toward establishment of definite centers of variability seems to be lacking except in those instances where geographical isolation of the host species provides opportunity for specific differentiation of the hosts which may then be accompanied by a parallel differentiation of the species of acanthocephalan parasites. Thus one avenue for augmenting species lists through elevation of sub-species to full specific rank, so obvious in many animal groups, is practically lacking in the literature on the Acanthocephala.

All of this which I have been narrating is an accounting of the problem of recognizing diversity in the Acanthocephala as achieved in the past. Permit me to glance for a moment to the future. Scores if not hundreds of species of Acanthocephala are as yet unknown. Considerable numbers of species regarded as valid by overenthusiastic workers will doubtless have to be sunk in synonymy. Several genera are of highly questionable status and a few of the families are in dire need of reconsideration. But each year a considerable number of new species and new genera find their way into the literature. There is every reason to expect this to continue for some time because there are many regions of the world where few samples have ever been taken and great numbers of potential host species which have never been examined for Acanthocephala. This phophecy of continued expansion of the field is a prediction based on sampling of incompletely studied collections, not a wild or over enthusiastic guess. In collections from the far corners of the earth which generous colleagues have submitted to me for study there are significant numbers of species waiting description. While most of the outline of the picture of the world fauna of Acanthocephala has been sketched, many of the details of the completed picture remain unfinished.

By way of summary of this section, the majority of the taxonomic descriptions of parasitic worms given by the early writers were entirely devoid of any details of morphology which would enable anyone at this date to recognize the species from the original description alone. This was particularly true for all the early descriptions of the species of Echinorhynchus while that genus was still regarded as embracing all of the thorny-headed worms. Esoteric information regarding localities and hosts furnished the chief basis for recognition of many of the species when original specimens were not available to later workers. Even then decisions had to be made upon wholly problematical bases when two or more species could equally satisfy all of the original description. Students of Acanthocephala are particularly

indebted to Max Lühe (1904–5) for his labor of love in making a critical review of all of the early specific descriptions. Many of his decisions were obviously arbitrary. Equally strong cases could be made for a claim of identity different from that maintained for some species by Lühe. Argumentation on such matters is meaningless except in cases where new evidence is uncovered, particularly in the form of specimens named by an early author. Even here there are so many opportunities for error in old collections and in relabelling of specimens that there is little opportunity for definitive conclusions. It has been the writer's policy to accept the claims of identity established for the early specific descriptions by any reputable worker of a later date if negative evidence against his decisions is not apparent.

Out from this background of confused concepts and inadequate descriptions at all levels of taxonomy, modern helminthology has emerged. Its progress is still impeded by uncertainties and errors which have never been and probably never can be corrected. Its heritage of headaches over errors of the past calls for resolutions to avoid inaccuracies and incompleteness in the future.

III. SIGNIFICANT DISCOVERIES AFFECTING TAXONOMY

As a basis for taxonomy, superficial likenesses could be replaced by fundamental comparisons only after considerable body of morphological data had been accumulated. The works of Leuckart, of his students and of his successors provided much of that initial basic knowledge for the Acanthocephala which culminated in the masterly monograph by Kaiser (1891–3) and that by Hamann (1891–5). These works presented for the first time the details of gross internal structure and cytological minutiae which were ultimately to become the basis for taxonomic work in this group. The two men were about equal in their abilities to observe details and to interpret structures but Hamann carried his observations to a level of comparison which was lacking in the studies of Kaiser. It is for this reason that the present speaker (Van Cleave, 1936: 202) has accorded to Hamann the distinction of being the founder of modern taxonomic groupings of species in the Acanthocephala.

As background of the discoveries of Hamann and of Kaiser, it must be recalled that the earliest observers appreciated no basis for distinguishing between the various groups of the parasitic worms. As the next step, the emphasis was placed so strongly on the uniformity of all members of any group, such as the genus Echinorhynchus, that likenesses were over-stressed and differences were ignored. It became the privilege of Hamann to evaluate for the first time differences in the Acanthocephala at a level above those available for separating species. All of modern taxonomy of the Acanthocephala is the direct outgrowth of the recognition by Hamann of three genera in place of the single genus Echinorhynchus which had been sufficient to satisfy all of his contemporaries. On the basis of morphological and cytological details in the construction of the proboscis and its receptacle and the structure of the body wall, Hamann recognized the genus Gigantorhynchus as distinctive of a separate family, the Gigantorhynchidae, the genus Neorhynchus as type of the family Neorhynchidae, and Echinorhynchus as restricted by the removal of the two foregoing concepts as type of the family Echinorhynchidae. The nomenclatorial history of his genus Neorhynchus (= Neoechinorhynchus) is long and involved but the distinction of recognizing the concept of the genus belongs unmistakably to Hamann. All modern taxonomy at the level of genera and families of Acantho-

cephala is but an expansion of the fundamental discoveries announced by Hamann in 1892 as the immediate outgrowth of his morphological analysis.

From the 90's onward the number of investigators who turned their attention to the Acanthocephala increased rapidly. Many of these new-comers to the field left relatively little imprint except for the small isolated bits of information added here and there and for the expansion of the species list for the world fauna. Max Lühe (1904–5, 1911) grasped the full significance of the morphological and taxonomic studies of Hamann and carried the program to new levels, often correcting the ill-advised attempts of his predecessors and of his contemporaries. Following the era dominated by the works of Lühe, many younger men began helping with the analysis of the Acanthocephala, inaugurating programs of research on other continents. It will be for another generation to evaluate objectively the contributions of these recent investigations.

Among the moderns in acanthocephalan studies, no one name stands out more vividly than does that of Anton Meyer. His program was built upon all the studies which had preceded him and yet he added generously to morphological interpretations which enabled him to fabricate a comprehensive monographic treatment of all the known Acanthocephala (1932–3). His studies on ontogeny have likewise furnished the soundest basis for understanding acanthocephalan development that has as yet appeared.

While the morphological basis for taxonomy still retains its place of prominence in routine classification of the Acanthocephala there is no doubt but that results of studies in serology, in physiology, and in genetics may at some future date become applicable to practical analysis in acanthocephalan studies. Antigen specificity which rests upon protein specificity has potentialities for use in taxonomy and phylogenetic studies which have as yet been but little developed.

IV. TENTATIVE GROUPINGS OF SPECIES

The history of generic concepts in the Acanthocephala is long and intricately involved. Only a few of the early attempts and main currents in the progress will be treated here. After it became customary to apply technical names, many of the species first observed were in error assigned to other groups already familiar to the scientists of that day. Throughout all of his writings Linnaeus was obviously oblivious of the status or even the existence of the Acanthocephala. Except for a few species assigned erroneously to other groups, the entire taxonomy and nomenclature of the Acanthocephala was post-Linnaean in its development. As early as 1727, Frisch referred to an acanthocephalan under the classical designation of Lumbricus, a name widely used for tapeworms and nematodes by scholars before Linnaeus warped it into a previously unrecognized usage for a genus of earthworms.

The name Echinorhynchus for a generic concept was first published by Müller in 1776 and was there accredited by him to Zoega. Its adoption by Gmelin (1790) and by Rudolphi (1802) evidently accorded it wide acceptance as a group name. Other early generic names had very uneven careers. Probably the most interesting example of a generic name proposed, then generally ignored, and finally revived at a much later date is that of the genus Acanthocephalus. Koelreuter proposed this name in 1771, five years before the name Echinorhynchus was first used, yet contemporary students and writers down to the time of Lühe (1904: 147) gave it no

favorable acceptance. Koelreuter mentioned no species for the forms which he encountered in the intestines of *Leuciscus rutilis* and *L. idus*. Lühe (1905: 329) admitted that the drawings which Koelreuter gave were highly diagrammatic but he expressed the belief that on grounds of body form and number and condition of the hooks the description and figures could be regarded as applying to *Echinorhynchus anguillae* of O. F. Müller. In consequence, Lühe revived the name Acanthocephalus which almost all of Koelreuter's contemporaries had failed to accept and conferred on it the status of a valid genus with *Acanthocephalus anguillae* (Müller) as type by subsequent designation. Without the addition of esoteric information this reclothing of the concept could not have been possible. For many of the other poorly defined and inadequately known genera and species there has been no protagonist to perform the function of validation which Lühe gave to Koelreuter's Acanthocephalus. Several other names proposed as of generic rank were almost immediately sunk in oblivion and have remained there. This was the fate of Haerucula of Pallas, 1760; Acanthrus of Acharius, 1780; Pseudoechinorhynchus of Goeze, 1782; Haeruca of Gmelin, 1790; and Proboscidea of Bruguière, 1791. Contemporary and later workers seemed to regard these with as little favor as they had accorded to the erroneous assignments of species of thorny-headed worms to Fasciola, Taenia, Ascaris, and Sipunculus by eighteenth century writers.

In fact, Rudolphi (1802, et seq) and most of the workers of his day and many in the following generations appear to have regarded all of the names other than Echinorhynchus as superfluous and conceived either ignorance or in error because the name Echinorhynchus seemed to satisfy all needs for the expression of the level of knowledge above the specific category. Regardless of size, or form, or host relationships, every species appeared to comply to a stereotyped plan of superficial organization embodied in the definition of Echinorhynchus. Adaptations to the conditions of life imposed upon these obligatory parasites had so reduced the number of avenues along which differences could be reflected that they were regarded as extremely uniform and monotonous in their makeup. Intimate details of essential structures had not yet been discovered and were to remain unknown, except for a few isolated observations, until the closing decade of the nineteenth century when Hamann and Kaiser revealed the wealth of morphological differences between species that had, prior to their time, been thought to be identical in structure.

Lack of information on details of structure affected not alone the recognition of genera but also influenced the interpretation of relationships of the Acanthocephala. From very early times these worms and cestodes had been somewhat confused in the minds of zoologists. Strictly on the basis of superficial external resemblance, Goeze (1782) regarded a Taenia (*T. haeruca* of Pallas) as closely related to Echinorhynchus by applying the new generic name Pseudoechinorhynchus to it. Goeze further saw in the hook-covered proboscides of Acanthocephala and tetrarhynchid cestodes a fundamental similarity which led him to regard the two groups within his concept of the genus Echinorhynchus. Bremser (1819) continued to propagate this error by regarding Tetrarhynchus as a genus of Acanthocephala. Judging from the extent of morphological information available to Goeze, this conclusion regarding relations of cestodes and acanthocephalans must have been based wholly upon superficial likenesses. Perhaps with some slightly greater appreciation of fundamental likenesses, Leuckart later (1848: 68) created the class Anenteraeti

to include the Cestoda and Acanthocephala as orders. Apparently no feature of agreement other than the lack of a digestive tract was behind Leuckart's (1848) union of these two groups. Zoologists, despite the weight of Leuckart's authority, were reticent about adopting this feature of his classification.

The dangers of relying wholly upon superficial resemblances in the taxonomy of the Acanthocephala were not ended with the inadequate comparisons of the early investigators. In 1850, K. M. Diesing made what is probably the most serious blunder in the history of the broader interpretations of this group. There are extenuating circumstances which soften the errors of the early writers who mistook acanthocephalans for cestodes or flukes. Only blindest disregard for fundamental structures could have permitted Diesing to place the Acanthocephala along with the gregarines in his order Aprocta. His interpretation of relationships seemed to rest upon superficial agreement of form such as satisfied Cuvier in formulating his system of classification.

When Rudolphi (1808) coined the name Acanthocephala he regarded the Vermes as a class, apparently accepting Linnaeus' allocation of this class and within it he regarded the "Vermes acanthocephala" as an order coordinate with "Vermes trematoda," "Vermes cestoda" and "Vermes nematoda," offering no attempt at closer groupings of the orders to show varying degrees of phylogenetic relationship.

From this point onward the history of the treatment of the Acanthocephala as a group became highly complicated. Forgetful of the thesis which Leuckart advanced, placing Cestoda and Acanthocephala as closely related and coordinated groups, the weight of opinion swung distinctly toward a belief that Acanthocephala and Nematoda are fundamentally related. This position, based almost wholly upon negative evidences, was shared by most of the leading zoologists even including Hamann whose investigations provided some of the soundest evidences for regarding the Acanthocephala as closely related to the Cestoda.

Many of the early taxonomists placed the Acanthocephala as a group of the Vermes or Helminthes without divulging any belief in phylogenetic relationships. Louis Agassiz (1857: 208), the prominent pre-Darwinian authority on animal classification in America, placed the Acanthocephali as an order of the class Helminthes within the Linnaean concept of Vermes. His lectures and writings were so convincing that for a time many American zoologists accepted his system.

Ultimately a conviction that Nematoda and Acanthocephala have much in common began to find expression in terms such as the "Nematelmia" of Vogt (1851) which Gegenbaur (1859) emended to its more widely accepted form—Nemathelminthes. Until very recently the majority of student texts in zoology and in parasitology have continued to regard the Acanthocephala as a subdivision of the Nemathelminthes, coordinate with Nematoda and Gordiacea. The revered Parker and Haswell (1897), on which such a large percentage of the past generation of zoologists were nurtured, adopted this system for these "formidable internal parasites" but admitted (p. 297) that "the affinities of all the classes of Nemathelminthes are very obscure, and the propriety of grouping them into a single phylum is extremely doubtful."

This treatment became almost universal among writers of text books. The chief variant in the theme was whether the Acanthocephala merited recognition as a class, a sub-class, or an order.

A relatively small number of thinkers came to sense the fact that the Acanthocephala introduced troublesome disharmony within the pale of the round worms. Yet lacking convincing evidence to justify any new realignment of the groups, some writers adopted the noncommital policy of placing them, with other troublesome groups of "uncertain affinities," in a dangling appendix at the close of the formally integrated series of phyla, either as a distinct phylum or in unspecified status.

The speaker (Van Cleave 1941) assembled the evidences for regarding the Acanthocephala as an independent phylum showing more intimate morphological and biological relationships with the Cestoda than with any other group of animals. Pearse (1942) was the first recent American producer of a student text to adopt this point of view.

One of the individuals directly responsible for securing recognition of the Acanthocephala as a phylum was Sir E. Ray Lankester through his Treatise on Zoology (1900) and his article on Zoology in the Eleventh Edition of the Encylcopaedia Britannica (1911). Earlier in his Tabular View of the Phyla of the Animal Kingdom, Lankester (1877: 441) had made no attempt to locate the Acanthocephala for in a footnote he listed several unallocated groups for which he stated "On account of their very dubious affinities and probably degeneration, no attempt is made in the above system to include the following forms."

Many of the European writers of the present generation have followed Leuckart (1848) or have slightly modified his system in which the Vermes of Linnaeus were regarded as essentially a phylum. Anton Meyer, in Bronn's Tierreichs (1932–3) adopted a system in which the Acanthocephala were regarded as a class within the Askhelminthes, along with Rotatoria, Gastrotricha, Kinorhyncha, Priapuloidea, Nematomorpha and Nematoda. In the review of the phyla in the initial volume of her "The Invertebrates" (1940: 33) Dr. Hyman advocated this same alignment for the Acanthocephala. However, when she came to prepare the section on classification of the animal kingdom for the 1947 revision of the Britannica she regarded the Acanthocephala as a distinct phylum and this point of view she is incorporating in the second volume of her "The Invertebrates" now in preparation.

There has been but little divergence of opinion as to the name which should be applied to cover the thorny-headed worms in the aggregate, whether that group be regarded as a phylum or as a class or in any other taxonomic category. Rudolphi's (1808) use of the term Acanthocephala has been very widely accepted. For some unknown reason Stiles and Hassall (1920) attempted to prejudice the zoological profession toward acceptance of Burmeister's Acanthocephali which was proposed in 1837, 19 years after the name given by Rudolphi had begun to be used. Priority has no function in establishing names at this level but the solid entrenchment of "Acanthocephala" in the literature has assured its permanency. It has the full support by students of nomenclature, even of those who advocate uniform orthography for terminations of each level in taxonomy.

Any attempt to trace in full detail the history of nomenclature for the subdivisions of the Acanthocephala would unduly prolong this address.

V. REVIEW OF PHYLOGENETIC GROUPINGS

The studies of Hamann (1891–1895) opened the gate and paved the way for the modern era in acanthocephalan taxonomy. He showed that superficial similarities

are often the result of convergent tendencies in structures which are fundamentally distinct one from the other. The three families which he recognized in 1892 became the pivotal points around which all more recent classification of the Acanthocephala has revolved. The significance of the Echinorhynchidae, Gigantorhynchidae, and Neorhynchidae of Hamann as concepts goes far beyond the nomenclatorial aspects of the recognition of three families. The three major subdivisions, first regarded by Meyer (1931) and by Van Cleave (1936) as orders, are essentially the three concepts of Hamann elevated to a higher category. There is danger of loosing sight of the significance of fundamental discoveries in following the intricacies of formal nomenclature. It would be of little value to attempt to trace all of the varied treatments of the subdivisions of the Acanthocephala which have appeared in the literature. Only a few as illustrations of tendencies will be mentioned here.

Lauro Travassos (1913 et seq.) working on the fauna of Brazil, made numerous contributions toward revealing the distinctive acanthocephalan fauna of South America. Practically all of his attention was directed toward the analysis at the level of species, genera and families. The number of concepts bearing his name as author or reviser testifies to the extent of his labors.

Southwell and Macfie (1925) recognized the Acanthocephala as an order with three suborders. Each of the latter was essentially one of the families of Hamann expanded by the addition of the large number of genera and families which had been recognized since 1892. Faust (1929) elevated each of the Southwell and Macfie suborders to full ordinal rank and for each proposed a new name, differing from the original only in termination.

On a very incomplete understanding of the group as a whole, Thapar (1927) proposed the recognition of three orders within which the natural groupings were sadly confused. Witenberg (1932) likewise recognized three orders, two of which he accredited to Southwell and Macfie while the third he accepted from Thapar.

Meyer (1931, 1932–3) proposed an elaborate and in the main highly consistent classification based upon a more thorough understanding of comparative morphology and biological data than had been available to any previous worker. He regarded the Acanthocephala as a class of the Askhelminthes and pointed out fundamental details of morphology and ontogeny as the basis for two orders, the Palaecanthocephala and the Archiacanthocephala. Throughout his system Meyer found it necessary to make frequent mention of exceptions for some genera and families to allow them to fit into his scheme. In 1936 the speaker recognized the inharmonious elements which Meyer had regarded as exceptions to his characterizations as furnishing the basis for a unified group to which the name Eoacanthocephala was applied as a third order coordinate with the two orders of Meyer. The system of Meyer, as thus emended, has been very widely accepted by recent workers in the field and has been adopted in several text books.

In a paper delivered before the First Pan-American Medical-Social Congress held in Havana, Cuba in December, 1946, the speaker proposed a new organization of the taxonomy for the Acanthocephala. In this system, which regards the Acanthocephala as an independent phylum, two classes are recognized (see Table 1). One of these, the Metacanthocephala, is a new concept under a new name to embrace the orders Palaeacanthocephala and Archiacanthocephala of Meyer. The second class, named the Eoacanthocephala, represents the elevation of the group by

that same name proposed as an order (Van Cleave, 1936). Within the Eoacanthocephala the Gyracanthocephala and the Neoacanthocephala, proposed as suborders, are elevated to ordinal rank.

VI. SUMMARY AND CONCLUSIONS

You have granted me the opportunity to narrate some of the things which have interested me in connection with my program of research. I have attempted to set forth in brief the gradual unfolding of the knowledge regarding the Acanthocephala. Starting with a few scattered observations on oddities whose true peculiarities were not recognized at first and for which no adequate names were available, I have shown how scores of these little-understood thorny-headed worms gradually came to be known. At first they were regarded as tapeworms, or flukes, or nematodes, or sipunculids—almost everything in the roster of worms except the distinctive group

TABLE 1.—*Tabular analysis of the characters available for recognition of the Classes and Orders in the Phylum Acanthocephala*

	Class Metacanthocephala (New)		Class Eoacanthocephala	
	Order Palaeacantho-cephala	Order Archiacantho-cephala	Order Gyracantho-cepahala	Order Neoacantho-cephala
1. Body size	Small to large	Mostly large	Small	Small
2. Habitat of hosts	Aquatic	Terrestrial	Aquatic	Aquatic
3. Main longitudinal vessels of lacunar system	Generally lateral	Dorsal & ventral or dorsal	Dorsal and ventral	Dorsal and ventral
4. Cement glands of male	Multiple 6 or less	Multiple usually 8	Syncytial	Syncytial
5. Trunk spines	Present or wanting	Wanting	Present	Wanting
6. Number of subcuticular nuclei	Numerous amitotic fragments or few highly branched	Few highly modified and elongated	Very few giant nuclei	Very few giant nuclei
7. Proboscis hooks	Radially arranged ventral often larger than dorsal	Spirally arranged. Ventral not larger than dorsal	Radially arranged symmetrical	Radially arranged symmetrical
8. Proboscis receptacle	A closed sac with 2 circular muscle layers	With a ventral cleft (except Moniliformis) one circular muscle layer	Closed sac one circular muscle layer	Closed sac one circular muscle layer
9. Ligament sacs	Ruptured	Persistent, dorsal and ventral	Dorsal and ventral	Dorsal and ventral
10. Nephridia	Present or lacking	Lacking	Lacking	Lacking
11. Embryonic membranes	Thin	Heavy sculptured	Thin	Thin

which they truly are. Then came the recognition that all of these odds and ends sequestered as interlopers in varied groups could be pulled together under one generic name, Echinorhynchus. While additional generic names were proposed by early writers, some even earlier than Echinorhynchus, they were often applied in ignorance or in gross misunderstanding of the characters on which they were supposed to be founded. For many years the apparently monotonous similarity in all species of Echinorhynchus obscured every attempt at recognizing any fundamental differences which might serve to differentiate between the ever increasing number of species assigned to this genus.

In 1802 Rudolphi suggested the name Acanthocephala as a name for a group of class or ordinal rank but the name was a hollow symbol because to most scientists of that day and even those of more recent times the boundaries were exactly coexten-

sive with the limits of the old, familar genus Echinorhynchus. For years the name Acanthocephala stood on the frontiers of taxonomy and nomenclature much like the signs at the outskirts of some of our larger western cities, indicating a claim for potential size and importance which could not be filled for another generation when new criteria of likenesses and differences became available to bolster the few external features upon which taxonomic descriptions were drawn. It was only as a much later development that the distinctions between Acanthocephala and Echinorhynchus became recognized when additional genera, some old and many new, were given a place alongside Echinorhynchus to fill the void within the larger group and to stake out territories distinct from the shrinking limits recognized for an emended concept of the genus Echinorhynchus.

Three men were largely responsible for ushering in a new era of acanthocephalan studies through their attack upon problems of finer structure and development of members of this group. Leuckart, through his own pioneer researches and as a catalyzer of programs of study, made several contributions. Two men, working independently, made extensive additions to an understanding of the Acanthocephala. J. E. Kaiser (1891–3) prepared a monograph which was a model of careful detail of structure. A particularly keen sense of relative values in comparisons and contrasts permitted Otto Hamann (1891–5) to perceive for the first time a diversity within the uniformity in structure which had blinded all previous students of the thorny-headed worms. He carried his studies beyond portrayals of structure in individual species and was the first to suggest a fragmentation of the old genus Echinorhynchus on consistent differences. His was the distinction of proposing three distinct families (Neorhynchidae, Echinorhynchidae, and Gigantorhynchidae) based upon fundamental differences in bodily structure.

With this as the first forward step in the revelation of a diversified grouping, the following half century witnessed great activity in recognition of new genera and new grouping at all of the varied taxonomic levels. Some of this activity has been amateurish, over ambitious, and ill advised. On the whole the result has been the unveiling of a portrait of a system in a completeness which gives little resemblance to the few sketchy lines representing the inadequately understood species of the earliest investigators.

In striking contrast to its humble beginnings, this new blue-print calls for the recognition of the Acanthocephala as a distinct phylum of the Animal Kingdom. Some of the early microscopists whom we would like to honor as prophets had, apparently in ignorance, thought of these worms as belonging to the same higher category as the tapeworms. In spite of the fact that recent details of information (Van Cleave, 1941) support this view it must be recognized that the early pronouncements were spoken in the darkness of ignorance rather than in the scintillating light of prophecy. The relationships of this phylum to the round worms, the accepted view of many earlier zoologists, has been relegated to the position of a historically important error in judgment with little more than the weak cohesiveness within the class Vermes of Linnaeus to support it.

As a phylum the specifications now call for two distinct classes each of which is resolved into two orders. Among these four orders more than eighty genera have been recognized, most of them on valid grounds. The approximately 400 valid species are distributed through about 20 families, some of which may be ultimately

reduced to synonymy. While a great number of the genera were first recognized as monotypic, additional species have been recognized for most of them until only about one-third of the genera are now represented by a single known species and more than half of these have been recognized as of generic standing for less than 20 years.

This review of the growth of the concept of the thorny-headed worms may be likened to the gradual resolution of details and revelation of relationships of the parts of a scene viewed first at the extreme limits of vision on the horizon and then modified for the observer as he approaches nearer to it. At first the concepts were dimly viewed without even recognition of species. As time has brought the picture nearer for closer inspection, distinct species have been readily distinguished in misty, tentative groupings of genera and families. Upon still closer observation the major outlines and finer details, previously vague, confused or lacking, have been filled in to provide a sharply focused image of a phylum in all of its ramifications of internal and external relationships. An integrated view of the thorny-headed worms available to the student of today lacks the indefiniteness of its first horizons. The indistinct, sketchy concepts of the earliest workers have been superseded by the image of a well integrated series of natural units at all levels of taxonomy from the individual to the phylum resulting from a new evaluation of features and affinities.

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POROCEPHALUS CROTALI HUMBOLT (PENTASTOMIDA) IN OKLAHOMA

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A study of 550 rattlesnakes taken in Cimarron, Comanche, Harper, Haskell, Kiowa, Latimer, Le Flore, and Murray counties, Oklahoma, revealed common infection by the pentastomid *Porocephalus crotali* Humboldt. Although rattlesnakes infected by this parasite were taken in Comanche, Latimer, Le Flore, and Murray counties, the heaviest infections were in specimens taken from hibernation caves located in the Wichita Mountains Wildlife Refuge in Comanche county. As many as 158 specimens were taken from the lung of a single host. No more than five have been recovered from single hosts taken away from the caves, and the rate of infection was found to be 8.0 per cent among those taken away from caves and 15.9 per cent among those taken from the hibernation caves. All hosts are *Crotalus atrox atrox* Baird and Girard except for one specimen of *Cortalus horridus* Linné which harbored one female specimen. A study collection is being deposited in the United States National Museum and in the Museum of Zoology, University of Oklahoma.

Humboldt described *P. crotali* in 1808, having taken it from the lung of *Crotalus durissus terrificus* Laur. collected at Cumana, Venezuela. It has since been reported from the following North American hosts: *Crotalus adamanteus* Beauv. by Leidy (1884), *Crotalus horridus* by Shipley (1898), from "a rattlesnake of undetermined species" by Sambon (1922), and *Crotalus atrox* by Hett (1924). Leidy's specimens were collected in Florida, and those of Sambon from an island in the Gulf of California. Shipley and Hett did not give the locality from which their records were obtained. The only other record of *P. crotali* in North America is that of larvae taken from the Louisiana muskrat by Penn and Martin (1941) and the adults from the cotton mouth moccasin by Penn (1942). Although the above records indicate that this parasite is distributed over a wide geographic area and that there is little specificity of hosts, accounts of it have been meager even when the foreign records are considered. Heymons (1935) listed only *C. terrificus, C. horridus, C. atrox* and *C. adamanteus* as hosts.

Specimens in the Oklahoma collection conform in many respects to the generic description given by Heymons (1935), but certain variations from this discription merit attention. A study of both living and preserved specimens has failed to reveal the presence of auxiliary hooks attached to the outside pair. Since this character has been considered a prominent generic one, the question of establishing a new genus or eliminating this structure in the generic designation must be considered. This would require a comparative study of the various collections, and since the authors have not had the opportunity to observe other collections, no attempt is made here to decide this question. Another point which warrants mentioning is that males in the Oklahoma collection show prominent intersegmental furrows through the mid region (Fig. 1) whereas previous descriptions indicate that these are indistinct.

It has been difficult to make a specific determination because, as Heymons (1935) pointed out, the three species, *P. crotali, P. clavatus,* and *P. stilesi* differ in only

Received for publication, October 30, 1946.

minor details, except for host records. Using specific characteristics compiled by Heymons (Table 1), it is seen that the three species are quite similar with reference to length and breadth of body, and number of segments. The reported variations in number of segments are probably due to the difficulty of counting them in the cephalic region, and the variations in size could easily be due to host conditions or degree of maturity. Specimens in the collection under consideration could well fit into either species from the standpoint of measurements and the number of segments.

TABLE 1.—*Summary to show comparative differences in length, segment number, and head width by sex of* P. crotali, P. clavatus, *and* P. stilesi *with Oklahoma specimens*

Species	Length, female	Length, male	Number segments, female	Number segments, male	Width of head, female	Width of head, male	Author
	mm	*mm*			*mm*	*mm*	
P. crotali ...	50–65	25–27	30–35	34–37	4.8–8	3	Hett
P. clavatus ..	75–86	25–36	38–44	35–43	No data	No data	Sambon
P. stilesi ...	80–96	30–38	42	43	5–6	3.5–4	Sambon
Oklahoma specimens .	60–70	30–35	39–40	40–41	7.8–9.2	3.1–4	....

The posterior segment in the females of our collection is club shaped with a conical point as described for *P. crotali* (Fig. 2) but is not markedly different from the posterior segment of *P. clavatus* figured by Heymons. The terminal segment of males in our collection is only slightly larger than the preceding ones (Fig. 1) and is more like that described for *P. stilesi.* Also, the anterior end of our specimens is not distinctly pestle shaped as described for *P. crotali* and *P. clavatus* but is more like that of *P. stilesi* figured by Heymons.

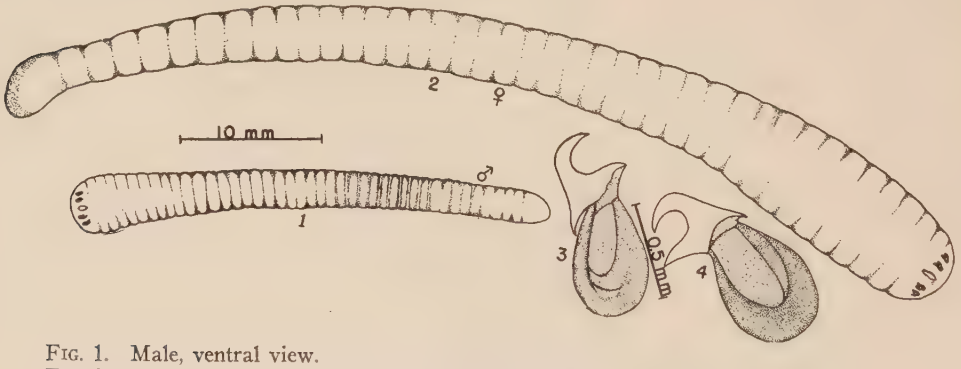

FIG. 1. Male, ventral view.
FIG. 2. Female, showing ventral view in anterior end and side view in posterior end.
FIG. 3. Right median hook.
FIG. 4. Left laterial hook.

The size of the hooks apparently have not figured prominently in previous descriptions of species of *Porocephalus.* In only one case does Heymons mention the measurements of hooks, that in *P. stilesi,* which Sambon gave as being 1.5 mm long. In the specimens taken from Oklahoma the hooks measure less than 0.5 mm in length from base to tip (Figs. 3 and 4). The base is broad and hinged to a horseshoe shaped fulcrum which is imbedded in the integument. The bases of the hooks are located in a depression so that only the tips are visible in undissected specimens. Fig. 3 shows a right median hook while Fig. 4 shows a left lateral one. They are alike in size and shape and cannot be distinguished as opposed to the unequal condition described for all other members of the genus.

The authors are in agreement with Heymons that there has been little to distinguish the three species, *P. crotali, P. clavatus, P. stilesi,* except for habitat. If the three should be combined, *P. crotali* having priority would become the established scientific name. The absence of auxiliary hooks in the Oklahoma specimens discussed in this paper seems to separate these specimens more widely from others previously described than the latter are separated from each other by any single morphological feature. Further comparative studies may warrant placing our specimens in a distinct species and eliminating the auxiliary hooks as a generic characteristic.

In an attempt to identify the secondary host, rodents were trapped in dens from which snakes had been taken. Of three woodrats and ten white-footed mice, one specimen of *Peromyscus leucopus aridulus* Osgood trapped March 30, 1947, was infected with ten larvae. These were located in the lungs, pancreas, and mesenteries. Since *P. lucopus aridulus* is one of the most common rodents frequenting dens in the Wichita Mountains, it is not surprising that it serves as a secondary host.

The ecological factors in the Wichita Mountains governing infections by this parasite both in rodents and rattlesnakes are interesting. The mountains are granite escarpments rising to several hundred feet above the floors of the valleys which separate them. Practically all dens are located near the tops of the mountains. It is known that the rodents and snakes which frequent these dens range over a very limited area. Our data reveal that in some dens no infections occur, while in others, the rate is over fifty per cent. This is due to the isolation of the inhabitants of different dens from each other by the valleys which separate the mountains. Also, since the rodents and snakes live in such close association in the dens, one would expect that once an infection is introduced into a den population by a rodent or snake it will build up in it, especially since the rodents are the principal source of food of the snakes.

SUMMARY

Rattlesnakes examined from Oklahoma reveal a high per cent infection of a pentastomid designated as *Porocephalus crotali* Humboldt. Specimens show size relationships with the three species of the genus *P. crotali, P. clavatus,* and *P. stilesi,* and differ from previous generic designations in the absence of auxiliary hooks and the sameness of lateral and median hooks. *Peromyscus leucopus aridulus* Osgood was found to serve as a secondary host. Infections are cumulative in snake and rodent populations in ecologically isolated dens in the Wichita Mountains.

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STUDIES ON THE LIFE CYCLE OF *LITOMOSOIDES CARINII*, FILARIID PARASITE OF THE COTTON RAT, *SIGMODON HISPIDUS LITORALIS*[1]

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THE INTERMEDIATE HOST AND EXPERIMENTAL TRANSMISSION OF THE FILARIAE INFECTING ANIMALS OTHER THAN MAN

Investigators of filariasis have been handicapped and limited in scope of study by lack of a suitable experimental animal which could be easily and relatively quickly infected with a filariid parasite. If such an animal could be found and experimentally infected, new fields of immunological and prophylactic drug studies would be open to research workers and chemotherapeutic studies on the age resistance of worms to various chemical agents could be made.

It is often assumed that the vectors of most species of filariid parasites are known. Witenberg and Gerichter (1944) state that there are 51 filariid species known from amphibians alone. Hundreds of filariid parasites have been described from both vertebrates and invertebrates. The vectors of these parasites are not known in most instances but only suspected on the basis of epidemiological data and a general knowledge of filariid vectors. Only about 15 of these hundreds of species of worms have been studied experimentally and have been shown to develop to presumably infective forms in certain arthropods (Table 1) but whether or not these arthropods actually serve as vectors in nature is unknown. Of this small group, transmission has been attempted with only five species. Two of these attempts were unsuccessful and three were probably successful although there is some uncertainty concerning two of the three experiments, and in the third no adults were ever recovered but four microfilariae were found.

Bancroft (1904) reported an experiment in which he probably infected a dog with *Dirofilaria immitis* through the bites of mosquitoes but failed to present evidence that the dog was protected from flea and mosquito bites before and after the experiment. Microfilariae appeared in the blood of the dog nine months after infected mosquitoes were permitted to feed upon it. More recent work by Brown (1939) and others has shown microfilariae may appear in the blood of puppies as early as eight months or less, so it is quite possible that Bancroft did succeed in infecting his experimental animal.

Received for publication, May 20, 1947.

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, under the Joint Committee on Graduate Instruction, Columbia University.

[2] A portion of the investigation described herewith was conducted while the author served as a Lieutenant H(S) USNR, assigned to the School of Public Health of the Faculty of Medicine, Columbia University, to aid in filariasis research.

I wish to express my sincere appreciation and thanks to the John and Mary R. Markle Foundation for Medical Research for the funds which made this study possible; to Dr. H. W. Brown, who suggested and supervised this problem; to the Hegener Research Supply Company of Sarasota, Florida, which permitted studies to be conducted in its laboratories; to the International Health Division of the Rockefeller Foundation for the mosquitoes, *Aedes albopictus* and *A. aegypti;* and to Miss Lucy Burbank, Miss Bettie Ferrell, Dr. Kathleen Hussey and Mr. Albert Rossi, for their technical assistance.

In an experiment somewhat similar to that of Bancroft, Bernard and Bauche (1913) attempted to transmit *Dirofilaria repens* to six puppies, utilizing *Aedes aegypti* as the intermediate host. Thirty days later, autopsy revealed traces of the mosquito bites but in none of the puppies were any worms found. This failure to find the immature worms might have been due to their small size, few numbers and the well known difficulty of finding worms at autopsy.

Pandit et al (1929) attempted to transmit *Conspiculum guindiensis* (*C. flavescens*) of lizards utilizing *Culex fatigans* (*quinquefasciatus*) as an intermediate host. They state that since all animals used in their experiment were caught in nature the possibility exists that they may have been infected prior to the experiment; however, they estimated, after a careful study of the percentage of infected lizards in the area from which they captured those used in their study, that only one of 21 animals used should have been naturally infected and four of the 21 were actually found to be infected following the experiment. They concluded that ". . . it can be

TABLE 1.—*Filariae infecting animals (other than man) and their suspected vectors*

Filariid parasite	Definitive host	Arthropod host in which development occurs	Successful lab. transmission?	Reference
1. *Conspiculum guindiensis*	Lizard	*Culex fatigans*	Probably—experiments not conclusive	31, 33
2. *Dipetalenema arbuta*	Porcupine	*Aedes* spp. *Taeniorhynchus* sp.	No	26
3. *Dirofilaria immitis*	Dogs	*Anopheles* spp. *Aedes* spp. *Culex* spp. *Ctenocephalides* spp. *Pulex irritans*	Probably—question of doubt exists	1, 22, 28, 34 6, 8, 42, 43
4. *Dirofilaria repens*	Dogs	*Aedes aegypti*	No	3
5. *Dirofilaria scapiceps*	Snowshoe hare	*Aedes* spp.	Yes	25
6. *Filaria cypseli*	African swift	Lice of sub-family Leiothinae	No	19
7. *Foleyella brachyoptera* *F. dolichoptera* *F. duboisi* *F. ranae*	Frogs	*Aedes* spp. *Culex* spp.	No	11, 12 13, 29, 49
8. *Icosiella neglecta*	Frog	*Forcipomyia velox* (Midge) *Sycorax silacea* (Sandfly)	No	18
9. *Onchocerca cervicalis*	Horses	*Culicoides nubeculosus*	No	38, 39
10. *Onchocerca gibsoni*	Cattle	*Tabanus* spp. *Culicoides* spp.	No	10, 15
11. *Onchocera gutturosa*	Cattle	*Simulium ornatum*	No	40

assumed that infection of the four animals was to a large extent experimental." Menon et al (1944) have shown that *C. guindiensis* will always produce microfilariae within 72 days when infective forms are injected subcutaneously. In one of the four infected animals of Pandit et al, microfilariae appeared on the 69th day after exposure to infected mosquitoes. This would appear to strengthen their case, that they probably experimentally infected at least one lizard.

Sometimes a presumed vector harboring infective stages fails to infect. Kotcher (1941) placed the labium of mosquitoes containing infective forms of *Foleyella brachyoptera* on the moistened web of the foot of a frog. The worms emerged from the labium but failed to penetrate the web or otherwise gain entrance into the frog.

Highby (1943a) experimentally transmitted *Dirofilaria scapiceps* from the snow-

shoe hare to the domestic rabbit through the agency of *Aedes fitchii* as an intermediate host. Two hundred and eighty-six days after infective mosquitoes fed upon the domestic rabbit, one microfilaria was found in the blood. Thirty days later a mosquito which was allowed to feed on this rabbit ingested one microfilaria. A postmortem examination 353 days after the attempted infection resulted in the finding of one microfilaria in a lung but no adult worms. In another experiment by Highby, three infective specimens of *Aedes cinereus* fed upon a rabbit. One microfilaria was recovered from the peripheral blood 391 days later. After 30 more days had elapsed postmortem examination of the animal failed to reveal microfilariae or adults.

In none of the preceding experiments was the success attained great enough to be useful from the standpoint of infecting large numbers of animals relatively quickly and easily in the laboratory. *C. guindiensis* developed rapidly in the lizard but it was difficult to infect as many as 20 per cent of the experimental animals. The lizard, a cold-blooded animal, might not be as satisfactory for certain studies as warm-blooded animals and it is very difficult to breed and maintain in the laboratory.

The length of the developmental time of the filariid parasites of dogs and rabbits, which approximates nine months, and the inability to obtain heavy infections, are serious handicaps for their use in laboratory studies.

Various subspecies of the cotton rat, *Sigmodon hispidus,* found commonly in some of the southern states, are naturally infected with the filariid parasite, *Litomosoides carinii* (Travassos). The adults of this nematode are usually found in the pleural cavity but have also been reported from the peritoneal space. The female parasites vary in length from 50 to 130 mm, while the males measure from 24 to 26 mm. The sheathed microfilariae, found in the circulating blood, measure from 70 to 90 μ exclusive of the sheath.

Naturally infected cotton rats have proved to be very valuable laboratory animals in investigations of chemotherapy of filariasis. However, cotton rats are trapped alive with considerable difficulty and since not all of them are infected the cost of infected animals is great. Furthermore, the age of the filarial infection in cotton rats is unknown which adds an unknown factor in chemotherapeutic tests. Since epidemiological evidence suggested that *L. carinii* developed rapidly to maturity within the cotton rat, it was thought that it might prove to be a useful parasite for laboratory use if transmitted to a suitable laboratory host. Since no arthropod vectors of this nematode parasite were known, this study was initiated in an effort to discover a vector and to attempt transmission of the infection to non-infected cotton rats and to the common laboratory white rat, which is more accessible, cheaper and easier to handle than the wild sigmodons. Results of the early work published by Williams and Brown (1945, 1946) have been confirmed by Hawking and Burroughs (1946), Bertram et al (1946a, 1946b) and Scott et al (1946). The present discussion gives in detail the complete study including the most recent findings.

EPIDEMIOLOGY OF FILARIASIS IN THE COTTON RAT, *Sigmodon hispidus litoralis*

The Host

The knowledge of the epidemiology of a disease is invaluable in ascertaining the arthropod vectors. Thus from epidemiological grounds alone, Maxcy (1926) hy-

pothesized the vector of endemic typhus to be a rat ectoparasite, and later work incriminated the rat flea, *Xenopsylla cheopis,* as the chief vector. It is logical, therefore, to secure as much data as possible on the habitat and habits of the cotton rat in order to ascertain all the arthropods to which it is exposed in the search for the vector of its filariid parasite, *Litomosoides.*

Habitats and Ecology.—The cotton rat, *Sigmodon hispidus litoralis,* abounds in the state of Florida, particularly in the western half. The area where this subspecies of cotton rat was studied and trapped lies on the west coast in Manatee, Sarasota, DeSota, Charlotte, Lee, and Collier counties.

Like the meadow mouse, the cotton rat is a creature of grasslands and overgrown open areas. It is dependent for its existence upon a thick screen of vegetation which protects it from hawks, owls and other predators. Throughout the region in which it was trapped, its well defined runways were everywhere evident under thick matted grasses, in sunflower fields, in areas of low ferns and briar patches and in salt-marsh grass. There was evidence that in these different types of habitats it relied upon the food available in each, such as roots of grasses, sunflower seeds, berries, fiddler crabs and large lubber grasshoppers, respectively. On occasion, the cotton rats dig holes and burrow in sand banks but usually they remain above ground. The young are born in round nests of grass built on the surface of the ground.

Snakes, hawks, owls, wildcats, racoons, muskrats, and alligators feed on adult and young cotton rats. Meyer and Meyer (1944) point out that it has been noted that the number of marsh hawks present in a given area about Thomasville, Georgia, is a good index of the number of cotton rats in that area.

According to Svihla (1929) the number of young per liter averages 4.75, ranging from 3 to 6. They are born hairy, weighing from 6 to 8 grams at birth and the eyes open within the first 24 hours. The young grow rapidly, about one gram a day and apparently are weaned at 10 days when they are able to feed on hard foods. He noted that the cotton rat is very prolific, producing young from early spring through late autumn. Stoddard (1931) found that while some young are born in the spring, the majority appear after June and the breeding season extends into the winter.

Accessory Factors (Age, Sex).—Rats trapped in non-infected areas were generally larger than infected rats and those who have examined thousands of rats at the Hegener Research Supply Company have noted that the largest rats are rarely infected. Laboratory experiments have shown that young rats become infected more quickly and perhaps more easily than do older rats.

Rats of both sexes are infected in about equal numbers in nature.

The Infection

The adult worms (described by Chandler, 1931) are found massed together in the pleural cavity but also occasionally inhabit the peritoneal space. The sheathed microfilariae are found in the circulating blood and measure approximately 70 to 90 μ in length excluding the sheath. The nuclei approach the tip of the tail. Bell and Brown (1945) have shown that these microfilariae do not exhibit a nocturnal or a diurnal periodicity nor a periodic cycle of longer duration, although there is a considerable variation in the number of circulating microfilariae from hour to hour and from day to day.

Geographical Distribution and Vertebrate Hosts.—Litomosoides carinii was first

described under the name *Filaria carinii* by Travassos in 1919 (Vaz, 1934) from the pleural and peritoneal cavity of a squirrel, *Sciurus* sp., from Sao Paulo, Brazil. It has been found in the rodents, *Holochilus vulpinus, Mus decumanus, Nectomys squamipes* and *Sigmodon hispidus*. It was first found in sigmodons in Texas by Chandler in 1931 and in 1932 was described by Ochoterena and Caballero from cotton rats in Mexico (Bell and Brown, 1945). The parasite has been reported from Brazil, Venezuela, Mexico, Texas, and Florida and from cotton rats in the latter three localities.

Incidence of the Infection.—The Hegener Research Supply Company of Sarasota, Florida, found that cotton rats were being infected throughout the year, in scattered foci, and that over a 17-month period, 43 per cent of all rats caught were blood positive for microfilariae. About 20 per cent of the rats which were considered negative and shipped from this area were reported to have become blood positive for microfilariae at a later date.

Pre-patent Period.—Most filariid worms, which have been studied, become sexually mature and pass microfilariae in from nine months to a year or more. The length of time required for microfilariae to appear in the blood of sigmodons after infection through the intermediate host, appeared to be relatively short, based on the following data. A blood-positive cotton rat caught in nature weighed 76 grams. According to Meyer and Meyer (1944) this weight should be attained within 50 days of birth in laboratory-bred rats. However, since development in nature may be somewhat slower than in the laboratory, the animal in question may have been 60 days old. The infection is not congenital; therefore, this sigmodon was infected after birth and the worms developed to sexual maturity within approximately 60 days. Later experimental evidence proved that the pre-patent period may, on occasion, be even less than 60 days but was found to be usually from 70 to 80 days.

The smallest worm which I have found in the pleural cavity of a sigmodon measured 1.112 mm in length. Since this length was only 112 μ longer than the infective stage found in the arthropod host, it is evident that the nematode reaches the pleural cavity soon after gaining entrance to the rat or that growth is very slow until the pleural cavity is reached. Cross and Scott (1945) state that a moult from the fourth larval stage to the adult stage occurs when the worms are between 7 and 10 mm in length, and Scott (1946) has found that microfilariae are produced when the worms are only about half grown.

Clinical Signs.—There are no clear-cut clinical signs which indicate the presence of this disease in the cotton rats. However, it has been observed that when trapping is done in a virgin area the highest percentage of infected rats is caught on the first night. Although the same number of rats may be caught on each of the two following nights, the percentage of infected individuals caught on the second night is less than on the first and less infected rats are trapped on the third than on the second night. This may indicate that infected animals may be less alert and content to take risks for their food, when it is easy to get, being lazy or too short of breath to search further.

Diagnosis.—The infection is detected by examining a drop of fresh tail blood for the active wiggling microfilariae or a dry blood smear stained with Giemsa. The microfilariae are readily seen under the low power of the microscope.

Infective Period.—An animal with filariasis is infectious to the intermediate host

and thus to other animals of its kind as long as microfilariae remain in its blood. In nature, infections may be superimposed one upon another so that actually once microfilariae are produced the sigmodon is never free of worms and will remain infective throughout its life. However, it is important for chemotherapeutic and other studies to know how long the adult worms live, when the peak of microfilarial production is reached, and how long microfilariae may be found in the circulating blood from a single infection or after all adult worms are dead. The increase and development of the microfilarial population within the infected cotton rat which was caught at the age of approximately 60 days was studied and investigations were conducted later with other animals. It was concluded from the study of these animals and their infections that adults of *L. carinii* may live approximately 60 weeks or more and reach their normal peak of microfilarial production between the 17th and 20th week after infection. Microfilariae may be found in the blood of infected animals after the death of the adult parasites for a period of time about equal to the time required from infection to the production of microfilaria, approximately 50 to 60 days. Greater concentrations of microfilariae were found in the heart blood than in the tail blood of infected animals.

Pathology[3]—Scott and Cross (1945) described tumors in the pleural cavity, probably formed as a reaction to dead worms or worms which had been injured and had adhered to the tissues of the host. Sections of these tumors, through which dead worms extended, showed that the tissue reaction was primarily a typical inflammatory reaction with proliferation of connective tissue. Culbertson and Rose (1944) reported that in rats treated with antimony compounds, dead adult worms recovered at autopsy, ". . . were held together in smaller or larger masses by a purulent necrotic exudate." I have found this condition to be present in untreated as well as treated rats. Whether the tumors found by Scott and Cross are the same as the masses of exudate described by Culbertson and Rose is not clear, but it appears that they may be.

Since the embryos are liberated in the pleural cavity, it seems probable that they must penetrate various tissues in order to reach the bloodstream. Harwood (1932) observed that burrowing is a normal activity for the sheathed microfilariae of *L. carinii*. He demonstrated their presence outside blood vessels in connective tissue and in the muscle of the diaphragm. He found that in muscle tissue the embryos usually lie parallel with the fibers and in no case were any found within a fiber. To confirm Harwood's work, to check on pathological conditions that might be initiated by such activity on the part of the young worms and to attempt to determine where they enter the blood stream, the lungs, heart, diaphragm, liver, spleen, kidney, salivary gland, and small intestines of a heavily infected rat were sectioned and stained with haemotoxylin and eosin for study. The shortest route to the bloodstream from the pleural cavity would appear to be through the lungs and through the heart. Indeed, of the organs studied, by far the greatest number of microfilariae were found within these two, with considerably more in the lungs than in the heart. In view of the fact that the numbers of microfilariae within the lungs far exceeded the number found within the blood of the heart it would appear that perhaps the lungs serve as the chief portal of entrance to the blood stream.

[3] Since this paper went to press Wharton has published an extensive article on the pathological changes in cotton rats with natural and experimental filariasis.

Microfilariae were found in considerable numbers between the myocardial fibers of the ventricles. Although their numbers and parallel location with each other within a given plane indicate that they may well be burrowing through this tissue, probably some were lying in capillaries. Many embryos seen among the cardiac muscle definitely lay outside of any blood vessels, sometimes lying across a fiber with ends tucked between that fiber and the two adjacent ones. None appeared to be within the fibers themselves. It seems likely that some microfilariae may reach the circulation by burrowing between cardiac muscle fibers.

The study of the diaphragm disclosed very few microfilariae and again it was difficult to decide whether the majority were burrowing between fibers or were actually in capillaries. The location of some left no doubt that they were outside of blood vessels and in a few instances they were found within the muscle fibers themselves (Plate I).

Relatively few embryos were found in the liver and spleen. Occasionally microfilariae were found in the glomerular capillaries of the kidneys and a few were lying between tubules. None were found in the small intestines or salivary gland.

It is evident that the sheathed embryos of *L. carinii* possess a decided ability to burrow between and into muscle fibers. The portal of entry of the microfilariae appears to be through the lungs and possibly the heart, although conclusive evidence is lacking.

Mode of Transmission.—This infection was presumed to be transmitted from one rodent to another through the agency of an haemophagous intermediate host. Basing deductions upon the behavior of other filariids, this vector should feed upon an infected animal and ingest a number of microfilariae during the course of the blood meal. These microfilariae, over a period of several days or weeks, should metamorphose and become infective. Should the infective intermediate host associate with an uninfected animal, the parasite may find entrance into the new host. It is the primary purpose of this paper to discuss the life cycle of this parasite which has been experimentally completed.

A STUDY OF POTENTIAL ARTHROPOD VECTORS OF *Litomosoides carinii*

All haemophagous arthropods may be considered as potential vectors of a disease involving parasites in the circulating blood. Our knowledge of the ecology of *Sigmodon hispidus litoralis* indicates that it is exposed to numerous blood-sucking arthropods. Representatives of this group were experimented with to test their ability to serve as a vector of *L. carinii*. Those considered in this study are listed in Table 2.

Winged Insects

Culicidae.—As indicated previously (Table 1), the majority of proved and suspected vectors of filariid worms are representatives of the Order DIPTERA, or flies, and more filariid species develop in members of the family CULICIDAE, or mosquitoes, than in any other family of the order. For this reason, and because they were more readily accessible than some of the other arthropods later studied, laboratory investigation began with this group.

A female cotton rat, having a microfilariae count of 3,875, by the technique described by Brown and Williams (1945), in approximately 3.5 cu mm of blood, and

her laboratory-born litter of two, which were negative for microfilariae, were placed in a large mosquito cage containing both males and females of *Aedes aegypti.* The colony of mosquitoes was then maintained for three months by feeding upon these cotton rats. At the end of that time the microfilaria count of the mother was 3,295 while the blood of the young rats was negative for microfilariae. Two months later the young rats were still found to be blood negative and they were autopsied. No filariid worms were found in the pleural or peritoneal cavities although sufficient time had elapsed for development to maturity.

In addition to *Aedes aegypti,* numerous other arthropods studied fed on infected rats. Table 3 lists the arthropods and number of each species which were dissected or sectioned at daily intervals following a blood meal on infected wild sigmodons. As indicated in Table 2, *Culex pipiens* L. was the only mosquito species which would not feed upon the cotton rat probably because the particular strain used was an autogenous one. Of the other five mosquito species studied (*A. aegypti, A. albopictus, A. taeniorhynchus, A. sollicitans, Mansonia perturbans*) 134 were dissected or sectioned serially with the result that no developing filariae were found in any of the

TABLE 2.—*Arthropods studied as possible vectors of* Litomosoides carinii

Arthropods	Suitability of cotton rat as host	No. microfilariae in stomach after blood meal	Comments
1. *Liponyssus bacoti*	+	0–53	.....
2. *Rhopalopsyllus gwyni* ...	+	?	None dissected immediately after blood meal
3. *Polyplax spinulosa*	+	8	.....
4. *Ixodes ricinus scapularis*	+	?	None dissected immediately after blood meal
5. *Aedes aegypti*	+	268–972	.....
6. *A. albopictus*	+	434	.....
7. *A. taeniorhynchus*	+	?	None dissected immediately after blood meal
8. *A. sollicitans*	+	?	None dissected immediately after blood meal
9. *Mansonia perturbans* ...	+	15,000–20,000	.....
10. *Culex pipiens*	–	...	Displayed no interest in feeding on cotton rat
11. *Chrysops* sp.	–	...	Readily caught and devoured by cotton rat
12. *Tabanus* sp.	–	...	Readily caught and devoured by cotton rat
13. *Cimex lectularius*	+	2,058	.....
14. *Culicoides furens*	–	...	Displayed no interest in feeding on cotton rat
15. *C. melleus*	–	...	Displayed no interest in feeding on cotton rat
16. *Culicoides* sp.	–	...	Displayed no interest in feeding on cotton rat

body tissues of any species. All microfilariae appeared to have been evacuated or digested between the third and fourth days, with the exception of one *A. aegypti* which contained 12 dead microfilariae in the stomach six days after feeding upon an infected rodent. Therefore, it was concluded that mosquitoes probably do not serve as intermediate hosts of *L. carinii,* although it is recognized that untried species of mosquitoes might be vectors.

Tabanidae and Ceratopogonidae.—Representatives of two other families of haemophagous DIPTERA, which are suspected as vectors of human filariids, collected in the natural habitat of the cotton rats, were investigated as being possible intermediate hosts of *L. carinii.* These included two members of the family Tabanidae, a species of horse fly, *Tabanus,* a species of deer fly, *Chrysops,* and three species of the genus *Culicoides* which is a member of the family CERATOPOGONIDAE.

When members of either species of TABANIDAE were released in a large cage with cotton rats, they were readily caught and devoured when they approached the rodents.

Several hundred sandflies, *Culicoides furens* Poey, *C. mellus* Coq., and *Culicoides* sp., were released in large cages with infected rats. Only one was found to have fed after three days. No microfilariae were found in this fly. The sandflies began to die rapidly four days after their release into the cage without evidence of having fed.

Because of the ability of the cotton rats to destroy tabanids and because of the apparent unsuitability of the cotton rat as a source of food for the midges, these two groups were considered as improbable intermediate hosts of the cotton rat filariid.

Non-flying Arthropods

A number of non-flying arthropods closely associated with the cotton rat were examined for their ability to become infected with *Litosomoides* (Table 2).

Cimex lectularius L.—The bedbug readily attacked cotton rats, although they were not found associated in nature, and as many as 2,058 active microfilariae were

TABLE 3.—*Result of dissections of arthropods following a blood meal on infected* Sigmodon hispidus litoralis

Arthropods studied which fed on infected cotton rat	Number dissected	Per cent in which development occurred	Comments
1. *Liponyssus bacoti*	82	1.2	82nd mite dissected had 14 developing filariae all about 500 μ in length
2. *Rhopalopsyllus gwyni*	137	0	No mf. found
3. *Polyplax spinulosa*	37	0	All mf. dead when examined
4. *Ixodes ricinus scapularis* .	7	0	All mf. dead when examined
5. *Aedes aegypti*	22	0	1 contained, in the stomach, 12 dead mf. 6 days after feeding on infected rat. In all others mf. had disappeared by 3rd day
6. *A. albopictus*	18	0	No mf. after 3rd day
7. *A. taeniorhynchus*	34	0	No mf. after 3rd day
8. *A. sollicitans*	28	0	No mf. after 3rd day
9. *Mansonia perturbans*	32	0	No mf. after 3rd day
10. *Cimex lectularius*	43	0	No mf. after 3rd day

counted from the stomach of one soon after feeding upon an infected sigmodon. However, as with the mosquitoes, no immature or developing filariae could be found in any of the tissues of some 43 bedbugs after the third day following an infective blood meal. Since the microfilariae were either digested or evacuated, it therefore appeared that bedbugs were unsuitable hosts.

Several groups of blood-sucking ectoparasites were commonly found on the Florida cotton rat. The louse, *Polyplax spinulosa* L., was frequently very abundant. The flea, *Rhopalopsyllus gwyni* C. Fox, could be found on nearly every rat in numbers from 1 to 17 with an average of about two or three per rat. The only tick found on these rodents was *Ixodes ricinus scapularis* Say. This was an infrequent parasite for it was only found, on an average, on every seventh rat of some 474 animals examined and usually only a single tick was present on a parasitized animal.

Six species of mites were collected from *S. hispidus litoralis,* by far the most common being the tropical rat mite, *Liponyssus bacoti* (Hirst), although *Atricholaelaps sigmodoni* Strandtmann was found in greater numbers on rats collected in the Everglades. The other four species were exceedingly rare and insufficient numbers were found to make determinations.

While conducting studies at the Hegener Research Supply Company of Sarasota, Florida, it was found that cotton rats born and raised in the laboratory became infected with *L. carinii*. An inventory of haemophagous arthropods found in the laboratory disclosed only mosquitoes and midges (*Culicoides*) other than the ectoparasites. Since these two groups of DIPTERA had previously been demonstrated to be improbable intermediate hosts of *L. carinii*, on the basis of unsuccessful laboratory infections, strong suspicion was cast upon the ectoparasite group.

Polyplax spinulosa.—Only dead microfilariae could be found in the mid-intestine of some 37 lice taken from blood-positive cotton rats. Eight microfilariae was the maximum number found in any one louse. This observation in conjunction with the belief that the natural biology of the louse would seem to inhibit it from being a good vector—since the majority of the lice spend their entire life upon the same host—led to the conclusion that lice were probably not responsible for the transmission of *L. carinii*.

Ixodes ricinus scapularis.—Seven ticks from blood-positive cotton rats disclosed only dead microfilariae in the gut upon dissection. The numbers of ticks found on cotton rats appeared to be far too low to account for the large percentage of infected rats and the high degree of infection found in the rodents. Ticks were, therefore, also considered as improbable intermediate hosts of the cotton rat filariid.

Rhopalopsyllus gwyni.—Eighty fleas of the species were collected from infected cotton rats, dissected and examined for developing filariae. These rats had been shipped to New York from Sarasota, Florida. In no instance were even dead microfilariae found, although Scott (verbal communication) has reported finding dead embryos of *L. carinii* in this species of flea. Likewise, 57 additional fleas examined at the Hegener Laboratory in Florida from blood-positive rats, were negative for filaria. It was therefore concluded that fleas probably do not serve as an intermediate host of *L. carinii*.

Liponyssus bacoti.—Mites, as well as fleas, had been collected from blood-positive rats shipped to New York from Florida. The dissection of some 54 mites disclosed either the absence of microfilariae or only dead microfilariae within them. However, further investigations on mites were made at Sarasota when it became evident that rats born in the laboratory were becoming infected and ectoparasites were therefore strongly suspected. Twenty-seven additional mites taken from blood-positive rats were examined with negative results. When the 28th mite, which was found crawling in the sawdust between two rat cages on a laboratory shelf, was dissected in a drop of saline, fourteen partially developed filariids, measuring approximately 500 μ wiggled free. This finding suggested that this arthropod might serve as an intermediate host and vector of *L. carinii* among the sigmodons.

Epidemiological evidence pointed strongly towards this mite as being the most probable intermediate host of the cotton rat filariid.

Rats were being infected the year around which would indicate that such arthropods as *Mansonia, Tabanus, Chrysops, Stomoxys* and possibly *Culicoides* were probably not the most important vectors since they occurred seasonally, being absent in the winter months.

Areas which contained only uninfected rats had mosquito and flea populations similar to the areas nearby from which infected rats were consistently trapped, indicating that the vector did not readily travel even relatively short distances.

The fact that increased rainfall appeared to lower the rat infection rate pointed to a host which was destroyed by rain, such as mite colonies on the surface of the ground, rather than one such as mosquitoes whose populations would be expected to increase with the rainfall.

Cotton rats collected in the Everglades had a low *Liponyssus* parasite index and a high *Atricholaelaps* index. The per cent of infected animals from this region was much lower and the degree of infection was less than in rats from other areas where *Liponyssus* was abundant.

The studies of haemophagous arthropods pointed to *L. bacoti* as the most likely vector of *L. carinii*. However, it is recognized that further investigation might incriminate some of the other species of which only relatively small samples were studied, or other species which were not investigated. Additional experiments to test *L. bacoti* as a vector of *L. carinii* were carried out.

THE TROPICAL RAT MITE, *Liponyssus bacoti* (HIRST)

Although Fonseca (1942) proposed a new genus, *Bdellonyssus,* for that of *Liponyssus* (*Liponissus*), the older, although perhaps not scientifically correct name, will be used here since through popular usage it is recognized by a majority of entomologists and parasitologists.

Hirst (1913) and Ewing (1923) described the adult male and female of this mite. Williams (1946) discussed the geographical distribution and reported previously unknown facts pertaining to the life cycle of *L. bacoti.* Bertram et al (1946b), have investigated the life cycle in detail and confirmed the work of other investigators that the larval stage does not feed. They have further shown that after feeding, the protonymph molts, changing to the deutonymph which, like the larval stage, molts without engorging and transforms into the adult. They also indicated that the life cycle is complete in 10 to 12 days at 25° C.

THE DEVELOPMENT OF *L. carinii* IN *L. bacoti*

Studies to Ascertain the Microfilarial Intake and Numbers of Infected Mites within a Colony

Upon finding the mite containing maturing forms of *L. carinii* in the Hegener laboratory in Florida, a colony of this arthropod was developed upon two blood-positive cotton rats. Mites from the colony were withdrawn at daily intervals and dissected, chiefly to ascertain the intake of microfilariae and the percentage of mites becoming infected. The first developmental form of the nematode in a mite from this colony (Colony A) was found 24 days after the colony was started. Table 4 gives the results of dissections of 190 of these mites taken at random over a two-week period beginning with the day a developing worm was first found in a mite from the colony. Nearly 14 per cent of these mites were found to be infected with developing forms of the parasite. It was later discovered that the period of time over which this colony was observed was not long enough for infective forms to mature within the mite.

Enough information had been obtained regarding the possibility of development in the mite to warrant transporting a colony to New York. Artificial rat nests were constructed and 837 mites collected and placed upon the rodents. Upon arriving in New York a fresh colony was started with 32 mites (Colony B) upon two positive

rats. Two weeks after the colony was initiated, mites were withdrawn and dissected and during the following month 500 were collected at random, dissected and studied (Table 4). Again about 14 per cent were found to contain developing filariae.

The per cent increase in length from microfilariae to infective forms is much greater for *L. carinii* than for other known filariid parasites. It was thought, therefore, that perhaps the time required for this development in the arthropod host would be somewhat longer for the cotton rat species, than for other species. *Wuchereria bancrofti* requires from 6 to 20 days to develop in the mosquito, depending for the most part upon temperature and humidity, but as a rule requires about two weeks. Since *L. carinii* increases in size about two and one-half times as much as *W. bancrofti* within its arthropod host, it was estimated that at least 30 days might be required for full development within the mite. Therefore, to determine the total developmental time, mites withdrawn from the colony started with 32 *L. bacoti* were carefully checked for infective forms. The first mite to contain a presumably infective form was found 37 days after the colony was started. Since this was not necessarily the first infective mite in the colony but merely the first one found by chance, it would appear that approximately a month might be required for full development. Bertram et al (1946b) have confirmed this estimate.

TABLE 4.—*Result of mite* (L. bacoti) *dissections*

Total number of mites dissected	Number with no microfilariae	Number with dead microfilariae	Number with active microfilariae	Number with developing filariae
Colony A 190	111 (58.4%)	42 (22.1%)	11 (5.7%)	23 (13.7%)
Colony B 500	286 (57%)	130 (26%)	12 (2.4%)	72 (14.4%)

Although the only source of food for these mites was the blood of highly infected cotton rats, it is interesting to note that about 58 per cent of all adult mites contained no microfilariae while nearly 24 per cent more contained only dead microfilariae. Bertram et al have demonstrated that filariae develop only in adult mites, possibly because the slow digestion of the deutonymph is unsuited to the development of the parasite.

A third colony (Colony C) was started by placing a few eggs of *L. bacoti* in the nests of infected rodents, and was allowed to develop for 45 days. At the end of this time large samples were withdrawn daily for a period of 28 days and each sample examined for mites containing only infective forms of the nematode. Of 4,093 mites examined 607 or 14.8 per cent contained fully developed larvae. A sample of 100 of the 607 infective mites was dissected and the fully developed worms counted. A total of 494 such worms were found or an average of 4.94 per mite. One mite was found to contain 56 worms, 23 of which were fully mature forms, 26 were sausage or early post-sausage forms and seven were only slightly developed.

The frequent and heavy infections among cotton rats in nature is quite understandable, for from this one colony of mites alone approximately 3,000 infective *L. carinii* larvae were observed and these represented but a fraction of the total number present.

Site of Development

In the mite, the embryos apparently burrow through the intestinal wall for small forms, slightly, if any, larger than microfilariae, were observed actively squirming

about outside of the digestive tract in the blood space. All developmental forms were also found in the haemocoele. The infective forms appeared, for the most part, to whip about actively without any great forward progress. At times, however, their motion would change and they could be seen to glide smoothly around and over the organs of the mite frequently entering legs and palps and then backing out.

Growth of L. carinii *within* L. bacoti
(Plate II)

At the time of ingestion with rat's blood the microfilariae vary in length from 70 μ to 90 μ and in width from 5.5 μ to 7 μ. In the mite, growth in length and width occurs and typical sausage forms 130 $\mu \times 16$ μ may be found within the haemocoele. According to Bertram et al (1946b) this size is attained in about one week. Size increase continued until a maximum width of 20 μ to 21 μ is reached when the worms are between 160 μ and 200 μ in length. While between 200 μ and 500 μ in length, the width remains at about 18.7 μ, then gradually decreases until the final width of 15.6 μ is attained when the length approaches 560 μ. It would appear from the observations of Bertram et al (1946b) that this final width is reached about the 22nd day of development. Growth in length then continues until the infective stages are formed between the 30th and 34th day.[4] The infective forms appeared to be of two definite sizes—800 μ, which may develop into males, and 1,000 μ which perhaps develop into females.

A 1,000 μ infective form was studied in detail and certain measurements taken. It was found that from the anterior end to the center of the most posterior portion of the nerve ring was 62.4 μ. From this point to the end of the pharynx was 35.8 μ. The esophagus was 282.2 μ in length and the genital anlage, which was 7.3 μ long, lay 41.3 μ behind the posterior end of the esophagus. From the center of the genital anlage to the anal pore was 539.9 μ and from the anal pore to the end of the tail 39 μ.[5]

THE TRANSMISSION OF *L. carinii* BY *L. bacoti* TO UNINFECTED RODENTS

Experiments on the Transmission by L. bacoti *of* L. carinii

The development of *L. carinii* in the tropical rat mite, *L. bacoti,* has been traced from ingested microfilariae through various degrees of metamorphosis to the presumably infective stage. To determine if this mite could transmit the infective forms to a new vertebrate host a series of experiments were conducted as follows:

Experiment I.—A colony of mites was developed on three white mice (laboratory reared and negative for filariae). Three weeks later the mice were removed from the colony and a blood positive female cotton rat and her young offspring, born in the laboratory two days previously, were substituted. Microfilariae were observed in a drop of blood from the tail of the young rat 101 days later. This period represents the length of time that was required for mites feeding upon the mother to become infected, the developing filariae to reach the infective stage within the mites, the mites to infect the young rat, and the worms to mature within the young animal to the stage where they produce microfilariae.

[4] In September 1947 Bertram stated that when his mites were incubated at 23–25° C transmission occurred as early as the 15th day after an infective feeding and as late as the 36th day.

[5] Bertram (1947) found that the length of a larva may vary even in the same drop of saline depending upon the location of the worm within the drop.

PLATE I

Microfilaria of *L. carinii* within a muscle fiber of the diaphragm of *S. hispidus litoralis.*

PLATE II

FIG. 1 Microfilaria of *L. carinii* from cotton rat blood. (70 μ × 5.5 μ)
FIG. 2 Pre-sausage form of *L. carinii* from *L. bacoti*. (94 μ × 8 μ)
FIG. 3 Sausage form of *L. carinii* from *L. bacoti*. (144 μ × 16 μ)
FIG. 4 Post-sausage form of *L. carinii from L. bacoti*. (260 μ × 18.7 μ)
FIG. 5 Pre-infective form of *L. carinii* from *L. bacoti*. (536 μ × 20.6 μ)
FIG. 6 Infective form of *L. carinii* from *L. bacoti*. (800 μ × 15.6 μ)
FIG. 7 Section of *L. bacoti* with developing *L. carinii* in haemocoele.

As a control to this experiment, two positive female cotton rats and their young, six days old, born en route from Florida, were placed in a similar nest without mites. Six months later one of these mother rats died. Several hundred worms were found in the pleural cavity. At this time her offspring was blood negative for microfilariae. At the same time the second mother rat in the control group had a microfilarial count of 2,463 while her offspring was blood negative for microfilariae. In both instances the young rats had been kept in the same cage with their respective mothers during the entire six months.

Experiment II.—A blood-negative pregnant female cotton rat was freed of all ectoparasites by wrapping in a chloroform soaked cloth and placed in an isolation cage. A few days later she gave birth to a litter of two, one of which died shortly thereafter. The mother and her remaining offspring were kept in isolation for three months. Both were blood-negative at the end of this time. A mite colony was developed on two heavily infected cotton rats and one month later the infected animals were removed from their mite colony and the blood negative mother and her young one (three months old) were substituted. The mite colony died out two weeks later. Seventy-eight days from the time these rats were exposed to the infected mites the young rat exhibited microfilariae in its blood while the blood of the mother rat was still negative. Autopsy of the young rat 200 days after the initial exposure disclosed 8 males and 17 females in the pleural cavity. The microfilarial count from tail blood at this time was 2,847 (Brown and Williams, 1945). Autopsy of the mother rat disclosed some evidence of a few worms having been present at one time, for two small masses of exudate, as described earlier, were found.

Two laboratory-reared cotton rats, four months of age, were used as a control to this experiment. During the time the other two animals were exposed to mites these two were kept together in a mite-free nest nearby. Neither exhibited microfilariae in tail blood one year later.

Experiment III.—A mite colony was developed on two heavily infected cotton rats. Two white rats 28 days old were placed in this mite colony. Ten days later one of them died. Autopsy resulted in the finding in the pleural cavity of six young worms ranging in length from 1.11 mm to 1.30 mm. The following day the second rat died and two worms were discovered, one 1.30 mm in length and the other 1.66 mm long. No worms were found in two control white rats of the same age which had been placed in a mite-free nest at the time that the two which became infected were placed in the nest with the mite colony.

Experiment IV.—Four laboratory-bred white rats, 30 days of age, were used in this experiment. Two of these animals were placed in individual wire-mesh cages, each cage being placed in a separate box beside a similar cage containing an infected cotton rat. A colony of mites was developed on each of the two cotton rats in their respective boxes prior to the introduction of the white rats. The position of the two cages in each of the two boxes was periodically interchanged so that the mites could more readily have access to the white rats after having fed on the infected cotton rats. The other two white rats, which were used as control animals, were placed together in a wire-mesh cage. This cage was placed in a box, which harbored no mites, beside a similar cage containing a cotton rat heavily infected with *L. carinii*. The position of the cages containing the control rats and the infected rat were also interchanged periodically. All three boxes, the two with the mites and the control box containing no mites, were kept in the same room.

The white rats subjected to contact with the mites were autopsied 42 and 44 days after being placed in the mite colonies with the infected cotton rats. The first white rat contained seven worms in the pleural cavity ranging in length from 1.165 mm to 12 mm while the second contained two worms, one 9 mm, and the other 42 mm in length. The two control rats were negative when autopsied after 44 days.

THE MECHANISM OF L. carinii TRANSMISSION BY L. bacoti

The question arises as to the manner in which the infective stage of the filariid parasite gains entrance into a new rodent host. Since they develop in the haemocoele of the mite, one wonders if their exit from the mite is oral or if, since their rodent hosts spend considerable time cleaning themselves, it is possible that infective mites are ingested and the rodents become infected in this manner. In an attempt to ascertain whether or not infection occurs by ingestion of infective mites or by the worms passing through the mouth parts of the mite while feeding, the following experiments were conducted:

Experiment I.—Three young white rats 32 days old were placed in a nest with two heavily infected cotton rats upon which a mite colony had been developing for 28 days. At the same time three other white rats, the same age, served as control animals and were placed adjacent to infected sigmodons in a nest without mites. One week later all white rats were removed and those that had been exposed to mites were freed of their ectoparasites. During this same week three white rats, 28 days of age, were each fed 400 live mites in saline by means of a rubber catheter. These mites were taken from colonies which had developed on blood positive rats for more than 30 days. Three other white rats were given saline, by catheter, without mites. Ten days later, 17 days from the beginning of the experiment, all twelve white rats were autopsied. Filarial worms were found only in the three rats which had been exposed to the bites of mites for one week. A worm 3.2 mm was found in the pleural cavity of one; eight worms, ranging in length from 1.33 mm to 5.83 mm were found in another and a single worm measuring 1.69 mm was found in the third. No worms were found in the rats fed infected mites, nor in the control animals.

Experiment II.—Throughout this and the following experiment only mites which were found to contain infective forms of the nematode were used. Eighty such mites, containing approximately 390 infective worms, were fed to a white rat approximately 60 days of age by means of a catheter. There was no indication of any worms in the pleural or peritoneal cavities when this animal was autopsied 37 days later.

Experiment III.—Fifteen mites containing infective larvae were placed on each of two young white rats about 30 days of age and 27 such mites were placed on another white rat of the same age. Two days later the nest of each was thoroughly searched for the mites. A total of eight mites were recovered, three of which no longer contained filaria, while the remainder harbored from one to six. The animals were freed of any mites that were attached to them and set aside for 35 days. Three white rats 30 to 38 days of age were each fed 15 mites containing infective filariid parasites. They also were set aside for 35 days. After this period had elapsed all six animals were autopsied. No worms were found in any of the three rats which were fed infective mites. Of the three rodents upon which infective mites had been

placed, one contained two female worms 37 mm and 51 mm long, and three male worms, one of which had been dead for some time; the other two males were 8 mm and 12 mm long. One mite free of infective forms of *L. carinii* had been recovered from the nest of this animal. The second rat in this series contained 12 male worms from 5 mm to 14.5 mm in length and two females both about 45 mm long. No mites had been recovered from the nest of this rodent. The third white rat from this group upon which mites had been placed contained one male of *L. carinii,* 11.5 mm in length. Two filariid-free mites were recovered from its nest.

The evidence presented here, although inconclusive, strongly suggests that *L. carinii* is introduced into a new rodent host through the skin rather than by ingestion. Only rodents upon which infective mites were placed exhibited maturing adults upon autopsy and, further mites which were known to be infective at the time of introduction upon the animals, were found, two days later, to be free of all worms.

It is difficult to comprehend how these infective worms, lying in the haemocoele of the mites, can escape without some injury to the mite, but such appears to be the case. Further investigation to elucidate the exact manner of introduction of *L. carinii* into the definitive host is necessary.

Susceptibility of the White Rat to Infection with L. carinii

It was hoped that the laboratory white rat which is more accessible, cheaper, and easier to handle, than the sigmodons, would prove to be a satisfactory host for the cotton rat filariid. However, evidence indicating that the white rat probably is not a satisfactory host is rapidly accumulating. The adults of *L. carinii* are known to live normally for 60 weeks or more in its natural host, the cotton rat. The length of life of the worms in the white rat is apparently much shorter, for in Experiment III above, a dead worm was reported from the pleural cavity of a white rat within 35 days of its introduction into the rodent. In no experimentally infected white rats were microfilariae observed more than 150 days after they first appeared in the circulating blood. Since microfilariae remain in the circulating blood of the cotton rat about 60 days after the death of all adult worms, it would appear that probably all adult worms in the white rats die by the time they are 90 days old or less. Bertram et al (1946a) confirmed this finding and stated: "although infective forms reached the adult stage, became sexually mature, and produced microfilariae, the adult worms in the pleural cavity of the white rat were progressively encapsulated and died within as short a period as 82 days of the first exposure to infection." Scott et al (1946) have also conducted experiments which suggest that previously uninfected white rats are not always as readily infected as similar cotton rats. They found that when two uninfected cotton rats and two similar white rats were exposed to the same mite colony at the same and for a similar length of time, the white rats harbored nine and 18 immature worms while one cotton rat harbored 106 mature worms and 898 immature worms and the other cotton rat contained 582 immature worms.

Hawking and Burrows (1946), using the mite technique developed by the author, have infected hamsters and mice in addition to white rats and piebald rats. In all cases, the results indicated that caution should be exercised before accepting the view that the presence of microfilariae in the peripheral blood of these animals necessarily implies that they are suitable hosts in which to establish a permanent strain of filariasis for chemotherapeutic or other studies.

It is notable that every instance of attempted experimental transmission to date has been successful; 100 per cent of all experimental animals became infected when subjected to infected mite colonies. The parasite develops rapidly in the cotton rat which is a natural host and satisfactory for laboratory studies, but unfortunately does not appear to live a normal existence in the white rat, an even more satisfactory laboratory animal.

SUMMARY

1. A survey of the literature shows that only about 15 of the hundreds of filariid parasites of animals other than man have been shown to develop to presumably infective stages within arthropods. Transmission experiments have been conducted with five of these species of which three attempts were probably successful although some question exists concerning two of these experiments and in the third, although a few microfilariae were found, no adult worms were recovered. It was evident that there was no known filariid worm which could be quickly and easily transmitted from one satisfactory laboratory animal to another. Consequently, investigators of filariasis have long been handicapped and limited in scope of study.

2. Various subspecies of the cotton rat, *Sigmodon hispidus,* are naturally infected with a filariid parasite, *Litomosoides carinii.* Since epidemiological evidence suggested that *L. carinii* developed rapidly within the cotton rat, it was thought that it might be a suitable parasite for transmission purposes.

3. The epidemiology of filariasis in the cotton rat was studied, including the habits, ecology and other factors pertaining to the sigmodons, as an aid in determining what arthropod might serve as a vector of *L. carinii.* The incidence of the disease in rats from Florida was found to be about 43 per cent. The infection, which occurred throughout the year, was found in scattered foci.

4. The infection in the cotton rat was studied. The prepatent period was ascertained to be 60 days or less on occasion, but usually between 70 and 80 days for most animals infected in the laboratory. The adult worms may live for 60 weeks or more in the pleural cavity and the peak of microfilarial production is reached between the 17th and 20th week. Microfilariae continue to be found in the circulating blood of infected animals approximately 50 to 60 days after the death of the adult worms. It is evident that the sheathed embryos have the ability to burrow between and into muscle fibers. The portal of entry of the microfilariae from the pleural cavity to the blood stream is thought to be through the lungs and possibly the heart.

5. The following arthropods were studied as possible vectors of *L. carinii: Aedes aegypti, A. albopictus, A. taeniorhyncus, A. sollicitans, Culex pipiens, Mansonia perturbans, Tabanus* spp., *Chrysops* sp., *Culicoides* sp., *C. furens, C. melleus, Cimex lectularius, Polyplax spinulosa, Ixodes ricinus scapularis, Rhopalopsyllus gwyni* and *Liponyssus bacoti.* Upon ingestion, the microfilariae appeared to be digested or evacuated in all but the tropical rat mite, *L. bacoti,* in which developmental forms were found. It is recognized, however, that further study might incriminate some other of these species, since relatively small samples were taken, or other species within these groups.

6. About 14 per cent of *L. bacoti* feeding on infected cotton rats contained developing filariae. Thirty or more days were required for complete development within the mite. Of some 4,093 mites taken at random from a colony which had

developed for more than 30 days, 14.8 per cent contained infective forms of the parasite. An average of 4.94 infective worms per mite were found in 100 infected mites examined. The development of the worm occurs within the haemocoele of the arthropods. The infective forms are 800 μ and 1000 μ in length by 15.6 μ in width.

7. Every attempted transmission experiment was successful when animals were placed in contact with infected mites. No experimental animals became infected when fed infected mites. This fact, and the fact that two days after mites containing infective worms were placed on rats, some were recovered which were free of worms, strongly suggest that *L. carinii* invades a new rodent host through the skin rather than by ingestion. Although white rats may be infected and microfilariae produced, the adult worms appear to live a relatively short time in this unnatural host.

8. It is now possible to infect readily large numbers of cotton rats (*Sigmodon hispidus,* an animal which may be successfully bred in the laboratory) easily and quickly with a filariid parasite, *Litomosoides carinii,* utilizing the tropical rat mite, *Liponyssus bacoti,* as intermediate host and vector, thus opening new fields of investigation in immunology and prophylactic drug studies and broadening the scope of chemotherapeutic studies by permitting investigation of the resistance of adult worms to various chemotherapeutic agents.

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THE BIOLOGY AND CONTROL OF THE FOX MAGGOT, *WOHLFAHRTIA OPACA* (COQ.)

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Among the many problems of parasitism confronting the greatly expanding fur-bearing animals industry in the United States and Canada, one that appears to have increased in economic importance because of the heavy losses caused among young foxes and mink, is maggot infestation by two sarcophagid flies, namely, *Wohlfahrtia vigil* (Walker) in the East, and *Wohlfahrtia opaca* (Coq.) in the West. The latter species has been called *Wohlfahrtia meigeni* (Schin.) in most of the literature, but, as we have previously shown (James and Gassner, 1947), *W. meigeni* is a European species.

The larvae of these flies produce myiasis in man as well as in the lower animals. Walker (1920, 1922) was the first to report such cases in infants, all five months of age or younger, and later the same worker (1931) recorded a total of eight cases. Brady (1923) and Chown (1924) described similar cases, and Ford (1936) reported parasitism in four babies, from three weeks to nine months of age. The pathogen in all these was *W. vigil;* however, *W. opaca* (recorded either as such or as *W. meigeni*) has been incriminated in cases reported from Montana by Mills, Callenbach and Reinhardt (1945), from Alberta by Morrison (1937), and from Utah by Rich and Knowlton (1937). Unpublished Colorado records of cases have been communicated to us by Dr. Edward R. Mugrage,[2] and what obviously was another case by Dr. C. E. Smith.[3]

It appears that the incidence of cutaneous myiasis in lower animals resulting from infestation by these two species is much more extensive than has been reported to date. *Wohlfahrtia vigil* was recorded as infesting rabbits and an Airedale puppy by Shannon (1923), rabbits by Johannsen (1926), and a fox puppy by Kingscote (1931). According to Kingscote (1935), the occurrence of the disease increased alarmingly in Minnesota in 1931 and in the Southern Ontario during 1934. The only apparently authentic record in an adult animal was found in a guinea pig by Knowlton and Hall (1941).

With the great expansion of the fur-bearing animal industry during the past fifteen years cutaneous myiasis has become more frequent among foxes and mink, and heavy losses in pups and kits are suffered at times by the fur farmers in certain areas. Knowlton and Hall (1941) stated that one farmer at Logan, Utah, lost 450 mink kits during two weeks in the spring of 1939 through infestation by *W. opaca,* and that individual losses among farmers in Utah and Idaho may amount to as much as $4,000.00. Winkler (1940), Ford (1941) and Schillinger (1941) discussed the seriousness of the problem arising from a seemingly greater abundance of *W. vigil* in the upper Mississippi Valley and the Great Lakes region. Gassner (1942, 1944) and Gassner and James (1946) reported in a similar fashion on the more frequent occurrence of *W. opaca* in the Rocky Mountain region, with a loss as high as 30%

Received for publication August 7, 1947.
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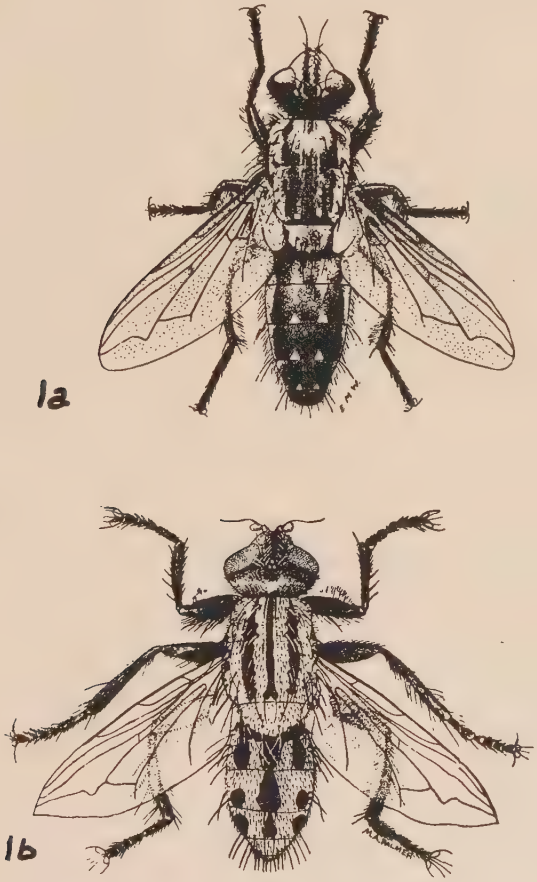
of the pup or kit crop on some of the ranches. Mills (1942) mentioned myiasis caused by this fly in mink kits in Montana, and through personal communications from various sources we know that similar trouble is being experienced in Western Kansas, Nebraska, and Washington.

Walker (1920, 1922, 1937) and Ford (1936) have dealt at length with the immature stages and biology of *W. vigil*. Our studies have been concerned entirely with *W. opaca*. Though similar in general in its biology to the eastern species, there seem to be several differences which will be discussed in this paper.



TEXT-FIG. 1a. *Wohlfahrtia vigil* (Walker) (after E. M. Walker). TEXT-FIG. 1b. *Wohlfahrtia opaca* (Coq.). (From original drawing by Mariam A. Palmer.)

The immature stages of *W. opaca* have been described by James and Gassner (1947). Several descriptions of the adult are available in publications, but adults of this species may be differentiated from other myiasis-producing species of *Wohlfahrtia* by the characters given in our key (James and Gassner, 1947) and from the related *Sarcophaga* by the tessellated abdominal pattern characteristic of most members of that genus. (Text-fig. 1a, 1b.)

The fly is widespread in the Western United States and Canada. We have records, either from the literature or based on the capture of specimens, of occurrence in British Columbia, Alberta, Washington, Oregon, California, Montana, Idaho, Utah, Wyoming, Colorado, New Mexico, Arizona, South Dakota, and Nebraska.

Records of maggot infestation, obviously by this species, add Western Kansas to the list. The known seasonal distribution based on specimens taken extends from late May to late September, but the presence of maggots at an earlier date indicates that the flies may be active as early as the last of March in protected valleys during early springs.

HABITS

Like *W. vigil,* the adult fly is not attracted to carrion or ill-smelling substances. Adults have been taken on flowers of onion, buckwheat, and miscellaneous native wild flowers. They are probably attracted by a rather wide variety of blossoms. Flies in captivity feed readily on cantaloupe, watermelon rind, honey, lump sugar, and dandelion blossoms. They have been taken in housefly traps baited with honey or molasses.

The food habits of adults may bear some relationship to infestations. Martin H. Muma (personal communications) mentions one instance in Custer County, Nebraska, in which an infestation occurring in fox pens with runways planted in rye was cleaned up when the rye was removed, and another at Hooper, Nebraska, in which mink in pens adjacent to an alfalfa field were infested. Any relationship between the infestation and the presence of the vegetation, however, is purely conjectural.

Light intensities seem to exert a limited influence upon the activities of the flies. Adults of both sexes will feed during the daytime, in full sunlight, according to data obtained from captured specimens in the field and observations of caged specimens. The darkness of the kennels seems to be no obstacle to larviposition on the part of the female.

The larva, like that of *W. vigil,* seems to be an obligate parasite, though apparently capable of living in the dead carcass longer than the Eastern species. Several fox pups from Delta, Colorado, which had died from maggot infestation, were shipped in dry ice to us in May 1946. From four to thirty maggots, in the second and third stages, were removed (Plate I, Figs. 1–3) from or observed in individual pups. Two heavily infested carcasses were placed in a natural temperature insectary for five days, at the end of which time the second stage larvae had died, but the third stage larvae were alive, subsequently pupated, and emerged as normal adults. In the field, in May 1946, a newly emerged adult was taken resting on the carcass of a dead fox pup. It is obvious from these facts that carcasses of animals that have died of myiasis should be destroyed to kill maggots that are sufficiently mature to complete their development in the carrion.

ATTEMPTS TO REAR THE FLIES AND TO PRODUCE PARASITISM

A colony of twenty flies, eleven males and nine females, was developed from third stage maggots obtained from fox pups from Aurora and Delta, Colorado. The maggots readily entered moist sand and pupated. Only one of twenty-two failed to emerge as an adult; one adult was accidentally killed. The larvae entered the sand on May 15 and May 17; the adults emerged from June 7 to June 12, the majority emerging on June 8 (8 individuals) and June 9 (9 individuals). One pair was observed in copulation on June 15; apparently no other mating took place. For some reason, the colony died over-night on June 23.

The experimental infection of an eight-week old rabbit was attempted on June 24 with maggots which had left the dead mother, but the attempt was a failure. Another

attempt to parasitize a rabbit was made with maggots which had hatched from eggs squeezed from the dead body of a female fly obtained from a bait trap. A four-week-old New Zealand white rabbit was selected for the test. The left dorsal lumbar region was shaved and the maggots were applied singly or in pairs to the bare skin. Data concerning the penetration test are summarized in the following table.

Maggot No.	*Time of Invasions*		*Minutes*
	Start	*Finish*	
1	3:20	3:30	10
2	3:42	3:52	10
3	3:42	3:52	10
4	3:46	3:52	6
5	3:48	3:52	6
6	3:55	4:06	11
7	No penetration, Weak		
8	No penetration, Weak		
9	No penetration, Weak		

Six out of nine maggots were able to penetrate the skin, as can be seen from the table, in an average of nine minutes. Stages of the invasion are shown in Fig. 6, Plate I. Twenty-four hours after penetration two of the lesions were distinctly visible (Fig. 7, Plate I) and the others had disappeared. No further enlargement of the lesions was noted during the following seventy-two hours. Upon autopsy six days after the invasions no trace of the maggots could be found. The sites showed normal repair and complete resorption of the larvae.

There were two conditions which may have proved greater obstacles to the successful negotiation of the skin barrier than would normally occur in nature. In the first place, the maggots were obtained under unusual conditions. Newly hatched maggots were used. They were not as active as others of the same brood were observed to be the following day. It is probable that the maggots are normally retained for a short period within the body of the mother before deposition takes place. In the second place, the rabbit was older than young animals usually attacked by the fly, and the skin was therefore more resistant. The rabbits reported by Johannsen (1926) as having *Wohlfahrtia* myiasis were about a week old.

Though the majority of parasitized foxes or mink that we have observed or which have been reported to us were less than four weeks old, there is evidence that adult animals also are parasitized. We have seen cases in adult foxes during an outbreak of distemper and on other occasions where resistance of young adults had been lowered and their movements restricted as a result of disease. These cases showed in every instance the typical lesions seen following the invasion of the unbroken skin.

THERAPEUTICS AND PROPHYLAXIS

Recovery of the young depends, of course, upon the degree of infestation. While the average number of larvae found in any one individual is about ten (one dead pup submitted to us was infested by only four maggots) we have recovered as many as sixty maggots from a single fox puppy. The timely removal of the larvae is usually followed by a rapid healing of the lesions. The application of either chloroform or ether to the openings in the skin causes the maggots to attempt to back out and this facilitates their removal with forceps. There is an urgent need for a thorough examination of every young animal on the ranch during the fly season as soon as

myiasis has occurred, regardless of the fact that this entails a considerable increase in labor to the fur farmer. Losses from myiasis will mount dramatically if control measures are not promptly and effectively applied when infestation occurs.

EFFECTS OF DDT

In preliminary experiments conducted in the spring of 1945 in cooperation with several fur ranchers, a dusting powder containing three per cent DDT in finely powdered talcum was tried as a control measure. The pups in every other row of kennels were dusted from a dusting can and the material was rubbed into the fur. The incidence of myiasis, according to the statement of the ranch operator, was 65 per cent greater in the untreated pups. In 1946 further trials were made. On one ranch considerable mortality from myiasis had occurred by the first week of May in pups less than four weeks of age. At this time maggots were removed from sixty-four pups, all of which survived. These and all remaining pups under four weeks of age were dusted with DDT with the exception of 10 which were considered too young (average 8 days old) to withstand the treatment. All of these untreated pups became infested with maggots. Six died and four recovered after removal of the parasites. None of the powdered pups developed myiasis.

Three weeks later, ten litters, comprising thirty-six pups, averaging six days of age, were treated with a preparation which contained 10 per cent DDT. None of these animals died following the application of this strong preparation, nor did any of them develop myiasis. In nearby kennels two recently whelped litters of pups were overlooked by the farmer. Six of these nine pups were found dead of myiasis at the time a routine check was made of the ten treated litters.

Another fur farmer reported to us that during May 1946, 117 out of 650 pups, or 18 per cent, were lost from maggot infestation. In accordance with our recommendation, the surviving animals were treated with DDT. Subsequent records kept by the rancher showed a loss of less than two per cent from myiasis during the months of June and July.

This information concerning the trials with DDT was obtained from cooperating fur farmers and verified by us whenever possible. It is at best only indicative that DDT has a value in the control of myiasis, since strictly controlled experimentation was seldom feasible in commercial operations. It is hoped that this phase can be investigated under controlled conditions during the present season.

As to the mechanism involved in the reduction of myiasis with DDT, it is probable that the maggots are killed while crawling over the hair to reach the skin of the host. Since DDT is not a fly repellent and since no dead *Wohlfahrtia* have been found in or near the kennels containing the treated pups, this seems to be the only explanation. The newly deposited larvae are very small and delicate and are perhaps susceptible at that stage to the insecticidal action of DDT. It has been observed by James (unpublished) during a city-wide fly control campaign in Fort Collins that more mature larvae of an undetermined *calliphorid* are not affected even when drenched with five per cent DDT solution in oil.

SUMMARY

1. The habits of *Wohlfahrtia opaca* (Coq.) are in general similar to those of *Wohlfahrtia vigil* (Walker). Adults are attracted to flowers and sweets. The flies may feed in direct sunlight, but females are not deterred from larviposition by the darkness of the kennels.

2. *Wohlfahrtia opaca* is an obligate parasite but third stage larvae are capable of maturing in the carcass of a dead animal.

3. Infestation results from the direct penetration of the unbroken skin by the maggots. Penetration may take place in ten minutes or less. As a rule only very young animals are parasitized, although adult animals when weakened by disease may be attacked.

4. Recovery is rapid, following timely removal of maggots. If infestation is heavy, or the condition of parasitism is allowed to continue, it will terminate fatally.

5. Three to ten per cent DDT, applied as a powder, seems to give results in preventing or reducing myiasis in fox pups

ACKNOWLEDGMENTS

The authors wish to thank Mr. Otto Zurcher, Aurora Fox Ranch, and Mr. Frank Morgan and Mr. Art Luckenbach, Longmont Fox Ranch, for their wholehearted cooperation in making this study possible.

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PLATE I

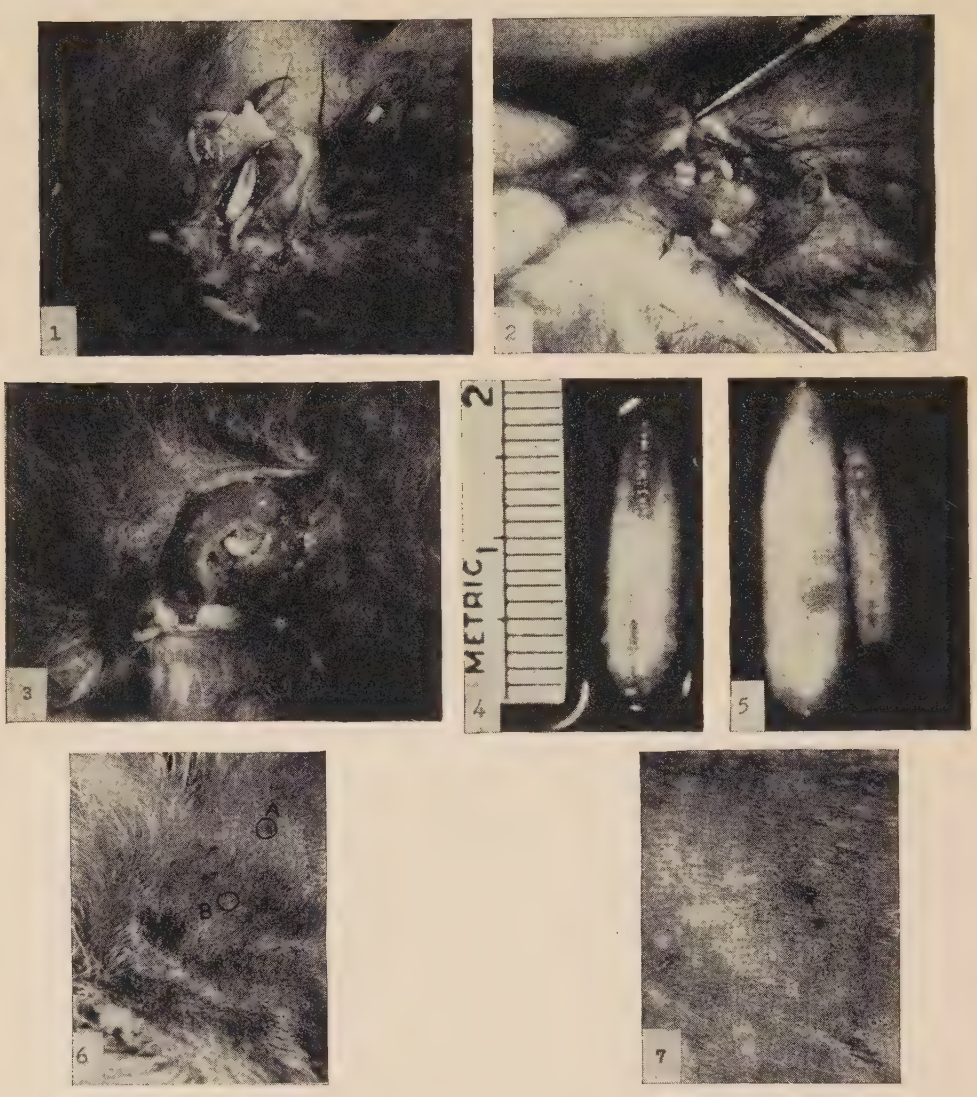

Fig. 1. Exposed flank region of a fox puppy dead of myiasis, with maggots of third stage in the subcutaneous tissue.

Fig. 2. The axilla exposed to show the deep invasion by several maggots of the second and third stages.

Fig. 3. Abdomen showing that maggots had penetrated the skin. A maggot is shown to be attached to the mesentery of a part of the small intestine. The invasion of any of the body cavities by these maggots has not been reported previously.

Fig. 4. Mature third stage larva, dorsal view.

Fig. 5. Mature third stage larva, lateral view (left) and a freshly moulted larva of the same stage, dorsal view (right).

Fig. 6. Shaved skin of rabbit, showing penetration by newly hatched first stage larvae. One larva (A) has completely penetrated the skin, only a reddened welt being visible; a second (B) is about two-thirds way in, only the posterior end being visible; and a third larva (right of B) is rearing upward. Three others may be seen crawling around.

Fig. 7. Same, 24 hours later. Two lesions made by invading maggots are visible.

OBSERVATIONS ON *NEOECHINORHYNCHUS EMYDIS* (LEIDY), AN ACANTHOCEPHALAN PARASITE OF TURTLES

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INTRODUCTION

The occurrence of a juvenile acanthocephalan in snails has been reported in a preliminary communication (Lincicome and Whitt, 1947). Reference was made there to the earlier work of Hopp (1946).

The worms were originally regarded tentatively as a new species because of the arrangement and character of the anterior proboscis hooks. In a restudy of adult *Neoechinorhynchus emydis* from turtles, the same hook pattern previously observed in juveniles from snails was observed. A further check of both juveniles and adults indicates that both represent the same species.

This paper presents observations on the biology and morphology of the juveniles of *N. emydis* with notes on the proboscis hook pattern of the adult worm.

THE JUVENILE OF *Neoechinorhynchus emydis* (LEIDY)

The juvenile form has been found encysted in the tissues of the foot, mantle, tentacles, and body of two species of snails, *Campeloma rufum* (Haldeman) and *Ceriphasia semicarinata* (Say), in the region of Lexington, Kentucky. Since only a few *C. semicarinata* have been studied, further remarks relative to infection in the snail host will concern *Campeloma rufum.*

The number of snails infected is apparently higher during late summer than in mid-winter and early spring, for on August 10, 1946, 72% of the snails were infected; on August 26, 1946, 59%; on October 23, 1946, 40%; on December 19, 1946, 33%; on April 27, 1947, 33% and on May 13, 1947, 54%.

In 75% of snails examined the worms were encysted on the right side, usually along the margin of the foot, or in the mantle near the anal opening. The cysts appear as opaque white tumefactions when the juvenile is viable, or yellowish or greenish-brown when the worm is dead.

There is usually but a single worm in each cyst, although on occasion there may be two. Commonly there are but 1 to 4 cysts per snail but as many as 19 have been recorded. Table 1 presents distribution of numbers of cysts of *N. emydis* in five collections of snail hosts.

Snail hosts ranged in size from 6 mm to 27 mm. Evidence (Table 2) indicates that a larger percentage of snails are infected with increasing size of shell and therefore with greater age. Although few very young snails were collected, there is a suggestion that snails may not often become infected before attaining a shell length of about 10 mm. Further evidence not presented here indicates that larger and therefore older snails, 20 mm or more, carry a greater number of cysts than snails below this size.

Van Cleave and Altringer, 1937, stated that the young of *Campeloma rufum* at

Received for publication, November 10, 1947.

birth have a mode in shell height of about 4 mm, and that the young snails grow rapidly from May, when born, to September, reaching a shell height in the 12 mm mode group. By May of the following year the shell has attained a height of 20 mm; the snail has reached maturity and bears young. If Van Cleave and Altringer's

TABLE 1.—*Percentage of* Campeloma rufum *having one to six or more* Neoechinorhynchus emydis *cysts in five host collections*

Collection Date	One cyst	Two cysts	Three cysts	Four cysts	Five cysts	Six cysts	More than six cysts
26–Aug–46	36	22	22	7	5	3	5
23–Oct–46	33	11	22	11	11	6	6
19–Dec–46	48	14	19	14	0	0	5
27–Apr–47	40	37	11	9	0	0	3
13–May–47	44	24	24	0	8	0	0

analysis of populations of *C. rufum* in East Central Illinois is valid for populations in the central Bluegrass region of Kentucky, this snail apparently does not become heavily infected with *Neoechinorhynchus emydis* cysts until it has approximated maturity.

Description of the juvenile of Neoechinorhynchus emydis—In preserved, mounted specimens: length and width of proboscis, 175–205 μ by 168–202 μ; body

TABLE 2.—*Percentage of* Campeloma rufum *infected with juveniles of* Neoechinorhynchus emydis *in relation to height of snail shell*

Shell height in mm	Total number snails examined	Average per cent infected
6–9	7	0
10–14	108	14
15–19	130	45
20–24	142	72
25–27	17	80

length, 1.2–1.7 mm; body width, 260–344 μ; length and width of proboscis sheath, 210–246 μ by 104–206 μ.

Anterior proboscis hooks six in number and of two types: longer, thicker type, of which there are two, inserted on the proboscis at a level posterior to the remaining four hooks that constitute the other type. The latter are all approximately equal in size and shape and are smaller than the former. Each of the greater hooks lies be-

TABLE 3.—*Measurements (in microns) of proboscis hooks of juvenile* Neoechinorhynchus emydis *as determined in whole mounts and living specimens from* Campeloma rufum

Hooks	Length		Width	
	Preserved and mounted	Living	Preserved and mounted	Living
Anterior:				
2 greater	108–113	106–117	31–34	34–44
4 lesser	82–99	83–104	23–28	23–33
Mid	52–62	55–67	9–11	10–15
Basal	45–56	45–56	8–10	8–11

tween two of the lesser forming a triangular arrangement that appears to be lateral rather than dorso-ventral. For measurements of all proboscis hooks see Table 3.

The two larger and thicker hooks set between smaller hooks in triangular fashion on the proboscis of this worm at first were considered distinctive enough to set the

animal apart from all other species of the genus *Neoechinorhynchus*. However, in a re-check of old mounted specimens of adult *N. emydis* there appeared to be a similar arrangement of hooks. Further study of fresh living adult material confirmed that the proboscis hook pattern observed in the juvenile form was identical with that in the adult.

PROBOSCIS HOOKS IN ADULT *Neoechinorhynchus emydis*

Living adult *N. emydis* were obtained from turtles purchased from a dealer in Arlington Heights, Illinois. The worms were placed in tap water and stored in the refrigerator until used.

The proboscis, below the level of the basal circle of hooks, was cut off and placed under a cover glass for microscopical examination. By withdrawing fluid from under the cover glass, the proboscis was compressed enough to bring hooks into profile for measuring. When this technic failed to bring them into suitable position, hooks were detached from the proboscis by manipulation of the cover glass. They were often thus isolated and brought into sharp profile for micrometric analysis.

Table 4 presents measurements of proboscis hooks on living *N. emydis*.

TABLE 4.—*Measurements of proboscis hooks (in microns) of living adult* Neoechinorhynchus emydis *from turtles*

Hooks	Length	Width
Anterior:		
2 greater	96–118	29–44
4 lesser	81–99	19–34
Mid	52–57	7–11
Basal	39–57	6–11

Previous descriptions of this worm (Van Cleave, 1913, 1919, and 1924), do not indicate any dimorphic condition of the anterior proboscis hooks, and give the following length measurements: anterior circle, 95–103 μ; mid-circle, 49–59 μ; basal circle, 35–54 μ.

Discrepancies between present and past observations on hook measurements may be explained on the basis of foreshortening of hooks due to faulty orientation in the optical field before measuring (Van Cleave, 1939).

DISCUSSION

The pattern of the anterior proboscis hooks here described for the juvenile and adult of *Neoechinorhynchus emydis* represents a departure from previous published records. J. E. Lynch in a letter to H. J. Van Cleave in April 1945 (personal communication from Van Cleave, March 1947) relative to *N. emydis* stated: "The terminal hooks in this species nearly always seem to have an irregular arrangement, some being decidedly anterior to others." R. M. Cable states (personal communication, January 1947) that in studies of *N. emydis* or a closely related species, "The lateral hooks of the anterior circlet are definitely set back of the ventral and dorsal hooks."

In a cursory examination of unidentified specimens of *Neoechinorhynchus* from Canadian fishes there seems to be every evidence that two types of hooks as in *N. emydis* form what has been called the anterior circle. Furthermore, two of the

hooks, as in *emydis,* appear to be set at a lower level on the proboscis than the other four.

That this same dimorphic condition in anterior proboscis hooks in *N. emydis* may be present in other species is suggested by the following statement from Lühe 1904–5 in a footnote to *Echinorhynchus clavaeceps* Zed.: "Die Angabe, dass *Ech. rutili,* O. F. Müll. (= *Ech. clavaeceps* autt.) 3 Reihen von je 6 Haken besitze, wie sie sich in gleicher Weise bei Dujardin (1845, p. 537, Nr. 64) und Hamann (Hamann 1891, p. 213, bez. 101) findet, ist allerdings insofern nicht ganz genau, als die 6 grossen Haken der hierbei als erste gazählten Reihe derart alternieren, dasz ich es für richtig halten würde, von 2 Querreihen grosser Haken zu sprechen, deren jede nur von 3 Haken gebildet wird."

SUMMARY

1. The juvenile of *Neoechinorhynchus emydis,* an acanthocephalan parasite adult in turtles, is described from two species of snails, *Campeloma rufum* and *Ceriphasia semicarinata.* Emphasis is placed on a form and arrangement of the anterior proboscis hooks herein reported for the first time.

2. Biological information relative to infection of the snail host is given.

3. A similar condition of anterior proboscis hooks is noted for adult *N. emydis* from turtles. A micrometric re-evaluation of proboscis hooks is given.

4. It is suggested that a similar arrangement pattern in proboscis hooks of other species of *Neoechinorhynchus* may exist.

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EVALUATION OF SELECTED INSECTICIDES AND DRUGS AS CHEMOTHERAPEUTIC AGENTS AGAINST EXTERNAL BLOODSUCKING PARASITES

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Lindquist, Knipling, Jones, and Madden (1944) demonstrated that bed bugs (*Cimex lectularius* L. and *C. hemipterus* F.) could be killed when fed on rabbits that had been given sublethal oral doses of DDT and pyrethrum. Some toxic action to stable flies (*Stomoxys calcitrans* (L.)) that had engorged on rabbits given pyrethrum was also apparent. The dosages required to produce insecticidal action in the blood of the host were relatively high, usually over 300 mg per kilogram of body weight. The results showed, however, that bloodsucking insects could be destroyed by internal medication of the host.

Recently De Meillon (1946) demonstrated that *Aedes aegypti* (L.), *Cimex lectularius,* and *Ornithodoros moubata* (Murray) were killed or affected when they fed on a rabbit given benzene hexachloride. Parman, Abbott, Culver, and Davidson (1928), Bruce (1940), Creighton, Dekle, and Russell (1943), and Emmel (1942) had previously been unable to demonstrate control of external parasites of animals by internal medication. Investigations by Knipling (1938) and Bruce (1939, 1940) demonstrated that phenothiazine and other chemicals fed to cattle would destroy larvae of the horn fly (*Siphona irritans* L.) breeding in the manure. Roark (1946), in reviewing some of the published work on internal therapy for plant and animal parasites, expressed a belief that this field of investigation offered promise for the development of effective agents that might be employed in this manner.

In June 1946 investigations were initiated by the writers at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine to determine the therapeutic value against several bloodsucking insects and arachnids of certain selected insecticides and drugs when administered to rabbits. The results of these investigations are herein reported.

MATERIALS AND METHODS

Rabbits were employed as the host animals in all the tests. Chemicals for testing were selected for the most part from among the more effective known insecticides, although a few chemicals in use as drugs were also tested. Most of the materials were first administered orally, either undiluted or dissolved in oil. The chemicals were usually administered at the rate of 300 mg per kilogram of body weight for the initial test. The compounds that showed some toxic action at this dosage were tested further at lower dosages.

Laboratory-reared body lice (*Pediculus humanus corporis* Deg.) and yellow-fever mosquitoes (*Aedes aegypti* (L.)) were permitted to take blood meals on the

Received for publication, December 12, 1947.

[1] The writers appreciate the suggestions and encouragement of E. C. Cushing, formerly in charge of the Division of Insects Affecting Man and Animals of this Bureau.

treated host. Culpepper (1946) had previously shown that body lice could be reared successfully on rabbits. Since some of the rabbits harbored natural infections of the ear mite (*Psoroptes equi* var. *cuniculi* (Delafond)), the effects of certain chemicals on these mites were observed. A few tests were conducted also against lone star ticks (*Amblyomma americanum* (L.)) that were allowed to engorge on treated rabbits.

The chemicals were administered to the rabbits in gelatin capsules, through a stomach tube or, in a few cases, by injection. Before the insects were fed, the host was tied down on its back in a V-shaped trough.

Lice, usually from 50 to 100, were placed on the belly of the host, from which the hair had been clipped. Lots of lice were usually permitted to feed at 6 and 24 hours after administration of the chemical and, if they were affected, at 24-hour intervals thereafter. The lice were permitted to feed for 20 to 30 minutes, and then 20 to 50 engorged lice were selected for observation. These lice were placed on cloth pads in 50-ml beakers and held in an incubator set at a temperature of 85°–86° F and in which the relative humidity ranged from 50 to 70 per cent. The procedure for handling the lice was similar to that described by Culpepper (1944). The effect of the chemical on the lice was observed at least once during the first 8 hours after the blood meal had been taken, and the mortality was recorded after 24 hours.

The mosquitoes were placed in 16-mesh cylindrical cages $2\frac{1}{2}$ inches in diameter and 10 inches long. One or two cages each containing approximately 100 mosquitoes were strapped lengthwise to the belly of the host in such a way that the mosquitoes could readily feed. The feeding schedule was the same as for the lice. After the mosquitoes had fed, they were chilled in a vault set at 38°–42° F, and the engorged specimens were selected for observation. The mosquitoes appeared unharmed by the chilling. Moistened cotton pads were placed on the cages to provide water for the mosquitoes. The cages were then placed in the same incubator in which the lice were held. The records for both insects were taken at the same time.

INITIAL TESTS WITH VARIOUS CHEMICALS

The results of the initial tests with the various chemicals are summarized in Table 1.

Results against Body Lice

Of 33 chemicals tested against lice, the following showed definite insecticidal action: gamma-benzene hexachloride; chlorinated camphene; chlordane; dicumarol (or 3,3-methylene bis(4-hydroxycoumarin); 2-caproyl-1,3-indandione; the potassium salt of 2-cyclopropyl-carbonyl-1,3-indandione; 2-enanthyl-1,3-indandione; 2-isovaleryl-1,3-indandione; sodium salt of 2-isovaleryl-1,3-indandione; 2-pivalyl-1,3-indandione; and powdered sabadilla seed.

Additional studies might have established definite toxic action with some of the other chemicals. However, in these tests the dosage employed was high, and it is probable that the materials which failed to show definite insecticidal action were relatively ineffective.

A number of chemicals proved fatal to the host at the dosage level of 300 mg per kilogram.

The only chemicals that caused complete kill of lice were some of the indandione

TABLE 1.—*Mortality of lice and mosquitoes 24 hours after they obtained one blood meal on rabbits receiving single doses of certain chemicals. Except where indicated otherwise, the undiluted chemical was administered orally at a dosage of 300 mg per kilogram*

Chemical[1]	Per cent mortality[2] on indicated days after administration of chemical to host															
	Lice								Mosquitoes							
	½ day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	½ day	1 day	2 days	3 days	4 days	5 days	6 days	7 days
p-Aminoazobenzene	7	5	3	..	..	..	..	..	0	0*	..	..	..	..	..	..
Atebrin	12	4	4	..	..	..	..	..	3	9*	7	..	..	..	..	..
Benzene hexachloride (gamma isomer)	8	6	16	8	20	..	12[3]	..	40*	86	100	100	100	100	100	..
	0	0	0[3]	..	..	..	..	..	60	100	100	..	..	..	..	..
Bisethylxanthogen	3	0[3]	..	..	..	..	..	..	6	0	..	..	..	..	..	..
n-Butyl mesityl oxide oxalate	0	0	0	..	..	..	..	..	0	0	3	..	..	..	..	..
Carbazole	2	3	6	..	..	..	..	..	8	0	0	..	..	..	..	..
Chlordane:																
Undiluted	4	8	..	..	16	..	..	..	4	0*	0*	..	2	..	..	..
10% in corn oil	8	12	60	..	..	..	..	..	20	54	76	..	99[3]	..	..	..
Chlorinated camphene:																
Undiluted	4	0	30	..	..	0	..	..	0	0	10	..	..	..	..	..
10% in corn oil (5 mg/kg)[4]	17	2	0	..	..	..	..	..	4	25	5	..	..	..	..	..
Chloromethyl *p*-chlorophenyl sulfone:																
Undiluted	17	0	0	0	4	..	..	..	6	3	19	59	39	4	..	..
5% in corn oil	17	8	0	..	..	..	..	..	31	97	23	15*	..	..	..	..
DDT:																
Undiluted	12	4	..	..	0	..	..	..	13	9	0*	..	11*	..	..	..
10% in corn oil	0	4	0	..	..	..	..	..	14	20	0	..	..	..	..	..
10% in olive oil (subcutaneous)	3	0	4	..	..	..	..	..	5	0	0*	..	..	..	..	..
2,4-Dinitro-6-cyclohexylphenol (100 mg/kg)	17	8	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1,4-Diphenylsemicarbazide	0	10	10	..	..	..	..	..	0*	10	0	..	..	..	..	..
Fouadin (intramuscular, 6.3% aqueous solution)	0[5]	[3]	..	..	..	..	..	..	2[5]	..	..	..	..	..	..	..
2-Hydroxy-2,4,4′,7-pentamethylflavan	0	12	..	4	..	..	..	..	2	0	..	..	..	..	..	..
1,3-Indandione derivatives:																
2-Caproyl	98[3]	..	..	..	..	..	..	..	6	..	..	..	..	..	..	..
2-Cyclopropylcarbonyl, potassium salt	70	100	100	58	48	..	0	..	36	94	78	85	8	..	..	..
N,N-Diethyl-2-butyrylamino, potassium salt	2	3	5	..	..	..	..	..	0	0	4	..	..	..	..	..
2-Enanthyl	98[3]	..	..	..	..	..	..	..	0	..	..	..	..	..	..	..
2-Isovaleryl	90	30	65[3]	..	..	..	..	..	9	58	18	..	..	..	..	..
2-Isovaleryl, sodium salt	100	100[3]	..	..	..	..	..	..	60	96	..	..	..	..	..	..
2-Pivalyl	100	100	100	100	100	100	100	100[3]	92	72	60	..	72	..	60	89
Isophorone	0	0	2	..	..	..	..	..	20	0	0	..	..	..	..	..
Mesityl oxide	2	0	0	..	..	..	..	..	4	0	..	..	..	..	..	..
3,3′-Methylene-bis-4-hydroxycoumarin (dicumarol):																
300 mg/kg	97[3]	..	..	..	..	..	..	..	25	..	..	..	..	..	..	..
100 mg/kg	23	95	86	30	10	0	0	..	10	0	41	53	8	..	..	..
Neoarsphenamine (intramuscular, 10% aqueous solution)	5[5]	[3]	..	..	..	..	..	..	10[5]	..	..	..	..	..	..	..
Phenothiazine	3	13	13	10	0	..	..	..	25	8	8	..	..	..	..	..
Phthalimide	0	0	0	..	..	..	..	..	0	4	0	..	..	..	..	..
Phthalonitrile (100 mg/kg)	3[3]	..	..	..	..	..	..	..	18	..	..	..	..	..	..	..
Pyrethrins:																
100%	4	0	..	..	..	..	..	..	0	0	..	..	..	..	..	..
50% (subcutaneous in olive oil)	6	4	0	..	..	..	..	..	6	2	0*	..	..	..	..	..
Pyrethrins (100 mg/kg) plus piperonyl cyclonene (200 mg/kg)	0	3	..	..	..	..	..	..	4	0	..	..	..	..	..	..
Rotenone	7	17	0	..	..	..	..	..	1	6	3	..	..	..	..	..
Sabadilla seed (ground)	28	20	8	..	..	..	..	..	2	0	..	..	..	..	..	..
Sodium fluoride (100 mg/kg)	10	0	4	..	..	..	..	..	0	0	0	..	..	..	..	..
No treatment	34 lots totaling 1,450 lice averaged 3.3% mortality (range 0–12%)								9 lots totaling 463 engorged mosquitoes averaged 3.2% mortality (range 0–8%)							

[1] Undiluted chemicals (crystalline or liquid form) were administered orally in gelatin capsules. Chemicals in oil solutions were administered through a stomach tube unless otherwise indicated.

[2] Figures marked with an asterisk (*) indicate that percentage mortality was based on less than 15 engorged individuals.

[3] Rabbit died.

[4] One rabbit receiving 300 mg/kg in corn oil died within 2 hours; another receiving 25 mg/kg died within 3 hours.

[5] Insects were given blood meals 2 hours after administration of the chemical for it was apparent that the hosts would soon die.

compounds. These compounds were tested further at lower dosages, and the results will be discussed later.

Results against Yellow-Fever Mosquitoes

Of the 31 chemicals evaluated against *Aedes aegypti,* the following showed some toxicity: gamma-benzene hexachloride; chloromethyl *p*-chlorophenyl sulfone; chlordane; dicumarol; potassium salt of 2-cyclopropylcarbonyl-1,3-indandione; 2-isovaleryl-1,3-indandione; sodium salt of 2-isovaleryl-1,3-indandione; and 2-pivalyl-1,3-indandione. Other chemicals may have caused some mortality, but the data are considered inconclusive.

Gamma-benzene hexachloride was the only material that killed all the mosquitoes.

Results against Ear Mites

On the rabbits harboring natural infestations of ear mites, observations were made on the effects of the following chemicals: pyrethrum; rotenone; DDT; 2-hydroxy-2,4,4,4′,7-pentamethylflavan; chloromethyl *p*-chlorophenyl sulfone; chlordane; chlorinated camphene; 2-pivalyl-1,3-indandione; and gamma-benzene hexachloride. Only benzene hexachloride killed the mites. Details of results with this chemical against mites are discussed in the section that follows.

FURTHER TESTS WITH GAMMA-BENZENE HEXACHLORIDE

Tests on Mosquitoes

Since mosquitoes were killed by blood meals from rabbits treated with gamma-benzene hexachloride at the rate of 300 mg per kilogram, further tests were made at lower dosage levels.

The material was administered orally in corn oil and in crystalline form, at dosages of 100, 25, and 5 mg per kilogram of body weight. Dosages as low as 25 mg per kilogram in both forms caused complete mortality of mosquitoes given blood meals after 24 hours, whereas the 5-mg dosage in crystalline form caused a maximum mortality of 93 per cent. The two animals receiving the 25-mg dosage died. It is believed that death due to such low dosages is unusual, especially when the chemical is administered in crystalline form. The animal receiving 100 mg per kilogram in crystalline form survived, and complete kill of mosquitoes was obtained for 5 days. Other animals employed in connection with further mite and tick tests survived dosages of 25 and 50 mg per kilogram.

An animal was given intravenously a 5 per cent solution of the chemical in Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate),[2] at a dosage of 1 mg per kilogram of body weight. Mosquitoes were fed at 40 minutes, 6 hours, and 24 hours after administration of the chemical. The mortalities after the feedings were 14, 12, and 5 per cent, respectively. Similar results were obtained with another host receiving about 1.4 mg per kilogram applied in the same way.

The mosquitoes feeding on rabbits dosed with benzene hexachloride were affected soon after they took blood meals. At dosage levels of 100 to 300 mg per

[2] Tween 20 was employed as a solvent for intravenous injections of chemicals subjected to pharmacological tests by the Food and Drug Administration. No adverse effects on rabbits were evident when this material was given alone at the rate employed in these tests.

kilogram some were paralyzed after 15 to 30 minutes and usually all were dead after 6 hours.

The results against mosquitoes are similar to those obtained by De Meillon (1946), whose data were published after these studies were completed.

Effect on Mites

Two animals that received 300-mg dosages of this chemical were heavily infested with ear mites. On the seventh day after the feeding, when one of the hosts died, all the mites were dead. The other host died on the third day, at which time from 50 to 75 per cent (estimated) of the mites were dead.

One animal which received the 100-mg dosage of gamma-benzene hexachloride in crystalline form, in connection with the mosquito tests, was lightly infected with mites. By the third day all the mites were dead.

Two heavily infested rabbits were each given a 50-mg dosage of crystalline gamma-benzene hexachloride. Within 4 days 50 per cent of the mites were killed, including practically all the adults. By the eighth day, however, the mite populations appeared to be developing normally.

Tests on Lone Star Ticks

Larvae and nymphs of *Amblyomma americanum* were placed on a rabbit one-half hour after the host had received 25 mg of gamma-benzene hexachloride (crystalline) per kilogram. Approximately 40 larvae and 25 nymphs attached within 16 hours. Most of the larvae and at least 14 nymphs engorged and were without apparent harmful effect.

Tests on Lice

The mortality of body lice allowed blood meals on rabbits receiving dosages of gamma-benzene hexachloride as high as 300 mg per kilogram (Table 1) was not high. When lice were permitted to feed at the same time on the same rabbits that were used in the tests with lower dosages against mosquitoes, the mortality was low, but the lice showed definite symptoms of toxic effect. These symptoms were evident after the lice fed on animals that had received as little as 25 mg per kilogram. Toxic action was rapid and the lice seldom consumed normal quantities of blood. Soon after the lice had fed, they became extremely active, and after 15 to 30 minutes some showed partial paralysis. Within 2 hours, however, they appeared normal.

FURTHER TESTS WITH 2-PIVALYL-1,3-INDANDIONE

Tests on Lice

Since complete kill of lice was obtained with 300 mg of 2-pivalyl-1,3-indandione per kilogram of body weight, further tests were made with smaller dosages. The results of these tests continued to show promise; consequently, this material and some of the other indandione compounds were given a rather thorough investigation. The various studies with 2-pivalyl-1,3-indandione eventually involved a consideration of (1) its effect on lice allowed one blood meal on rabbits that had received single oral doses, (2) the relative effectiveness of different methods of administering single doses, (3) its effect on lice allowed one blood meal on animals that had received single daily doses for several days, and (4) its effect on lice allowed several

blood meals on animals that had received single daily doses for several days. A discussion of the results of these studies follows.

(1) *Effect on lice allowed one blood meal on rabbits that had received single oral doses.*—The results of these studies are shown in Table 2. As may be noted, dosages of 300, 100, 25, and 5 mg per kilogram of body weight caused 100 per cent kill of lice, and the toxic agent persisted in the blood for several days. There appeared to be little correlation between the duration of effectiveness and dosages between 5 and 100 mg. In fact, on one rabbit (No. 63) receiving the 5-mg dosage the results were comparable to those obtained at the 100-mg dosage (No. 4).

When the chemical was administered at the rate of 2.5 mg per kilogram, the mortality of lice was still near 100 per cent at least on one day, but there was a definite reduction in mortality and also a shorter period of effectiveness. The lice surviving during the first few days were nearly all males, although no detailed sex records were maintained.

A dosage of 1 mg per kilogram is near the nontoxic level for lice receiving one blood meal, although in three tests some lice were killed. Since the 2.5 mg dosage proved to be near the minimum lethal dose, it is apparent that a dosage-mortality curve would be very steep.

(2) *Relative effectiveness of different methods of administering single doses.*—In these tests the material in solution was administered orally, subcutaneously, intramuscularly, intravenously, and by inunction to the unbroken skin. The dosage was 2.5 mg per kilogram. The results are shown in Table 2. There is apparently no great difference in effectiveness of the various methods of administration except the inunction method, which gave erratic results.

(3) *Effect on lice allowed one blood meal on rabbits that had received single daily doses for several days.*—To determine whether repeated doses of 2-pivalyl-1,3-indandione would accumulate in the blood, two animals (Nos. 38 and 30) received the chemical by forced feeding (in capsules), and two others (Nos. 70 and 69) were given the chemical with their food. Each animal was given a different dosage for a different number of days. Body lice were allowed one blood meal before the rabbits were fed and daily thereafter until the mortality decreased to a low point. Different lots of lice were used for each meal, and the mortalities were determined 24 hours afterwards.

Host No. 38 received 1 mg per kilogram daily for 3 days. The chemical in the form of a powder (1 per cent in pyrophyllite) was administered orally by capsule. Lice were allowed one blood meal each day for 19 days. From 25 to 50 lice were used each day. The mortality of lice before the animal was fed was 3 per cent. The daily mortalities after the initial feeding were as follows (no record on 4th day):

Day	Per cent	Day	Per cent	Day	Per cent
¼	4	7	84	13	68
1	28	8	88	14	44
2	94	9	85	15	56
3	96	10	72	16	56
5	84	11	73	17	38
6	84	12	70	18	64
				19	24

Host No. 30 received 0.25 mg per kilogram (in capsules) daily for 6 days. The animal was also used in connection with repeated blood-meal tests, as will be dis-

TABLE 2.—*Mortality of lice 24 hours after they obtained a single blood meal on rabbits receiving various dosages of 2-pivalyl-1,3-indandione administered in different ways*

Dosage mg/kg	Formulation	Method of administration	Host No.	Per cent mortality of lice fed[1] on indicated days after administration of chemical to host												
				¼ day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
300	Crystalline	Oral	7[2]	100	100	100	100	100	100	100	100	[3]	..	..	..	..
100	do.	do.	4	100	100	100	100	100	100	100	100	100	96	50	23	..
	10% in corn oil	do.	17	100	100	100	100	97	100	98	96	95	[3]	..	..	..
25	10% in Tween 20	Subcutaneous	1A	100	100	100	..	100	96	100	100	100	100	100	100	[3]
	Crystalline	Oral	23	100	100	100	100	100	100	100	100	80	50	47	..	12
	10% in corn oil	do.	24	100	100	100	100	100	100	62	17	14	..	..	..	..
5	Crystalline	do.	33	100	100	100	100	96	52	30	..	..	..	..	..	..
			63	100	100	100	100	100	100	100	100	95	93	71	60	20
2.5	1% in pyrophyllite	do.	39	20	92	98	93	..	100	52	50	50	36	8	..	..
			40	86	96	86	..	36	14	..	..	..	..	..	..	..
	1% in Tween 20	do.	43	96	96	69	68	47	12	..	..	..	..	..	..	..
			52	96	92	84	72	73	25	8	..	..	..	..	..	..
		Subcutaneous	45	95	100	100	96	100	96	62	27	4	..	..	..	..
			49	88	100	100	92	76	16	0	..	..	..	..	..	..
	1% in corn oil	do.	55	51	100	40	60	76	30	24	..	..	..	..	..	..
	1% in dimethyl phthalate	do.	56	38	96	52	60	36	6	..	..	..	..	..	..	..
	1% in Tween 20	Intramuscular	46	84	88	80	100	100	84	76	72	16	..	..	..	..
			51	87	88	80	63	12	..	..	..	..	..	..	..	..
		Intravenous	47	92	92	91	98	89	63	44	24	0	..	..	..	..
			50	100	100	100	100	88	56	53	75	20	17	..	..	..
		Inunction	44	..	4	0	..	..	..	..	..	..	..	..	..	..
	1% in dimethyl phthalate	do.	48	..	68	93	73	20	13	..	..	..	..	..	..	..
			53	0	4	4	..	..	..	..	..	..	..	..	..	..
1[4]	1% in pyrophyllite	Oral	35	2	10	12	20	[3]	..	..	..	..	..	..	..	..
	0.25% in corn oil	do.	36	4	18	7	6	..	..	..	..	..	..	..	..	..
	1% in pyrophyllite	do.	89	2	25	30	17	3	..	..	..	..	..	..	..	..

[1] Of 19 check lots (totaling 497 lice) the mortality ranged from 0 to 12% with an average of 2.8%.
[2] The data for this host are shown also in table 1.
[3] Rabbit died.
[4] 5 mg/kg of dicumarol given at same time.

cussed later. Lice were allowed one blood meal each day for 14 days. From 40 to 100 lice were fed each day. The mortality before the animal was fed was 3 per cent. The daily mortalities beginning 3 days after the initial feeding were as follows:

Day	Per cent	Day	Per cent	Day	Per cent
3	34	7	70	11	25
4	63	8	44	12	30
5	75	9	52	13	6
6	58	10	43	14	5

Host No. 70 was given food (rabbit pellets) containing 10 parts per million of 2-pivalyl-1,3-indandione for 19 days. The food was treated with the chemical dissolved in acetone, after which the solvent was allowed to evaporate. Each day 60 grams was offered to the rabbit, providing a daily dosage of 0.25 mg per kilogram. Only 45 grams was eaten the 1st day, but the 2nd day the animal consumed the daily ration of 60 grams plus the 15 grams remaining from the previous day. Thereafter, all treated food was taken each day plus about 30 grams of untreated food. Lice were allowed single blood meals each day for 26 days. From 30 to 50 lice were fed each day. The mortality of lice before the animal was fed was zero. The daily mortalities after the initial feeding were as follows (no records on 2nd, 3rd, and 25th days):

Day	Per cent	Day	Per cent	Day	Per cent
1	5	11	78	19	84
4	5	12	92	20	90
5	3	13	84	21	33
6	40	14	82	22	63
7	65	15	69	23	15
8	70	16	77	24	15
9	72	17	92	26	0
10	60	18	76		

Host No. 69 was also given treated food for 19 days. A daily dosage of 0.1 mg per kilogram was administered by offering the rabbit 50 grams per day of food containing 5 parts per million of the chemical. This host was also used to determine the effects of repeated feeding of lice, and the data obtained will be discussed later. Lice were allowed single blood meals for 26 days. From 30 to 50 lice were used in each test. The mortality of lice before the animal was fed was 5 per cent. The daily mortalities beginning 3 days after the initial feeding were as follows (no records on 6th and 25th days):

Day	Per cent	Day	Per cent	Day	Per cent
3	6	11	17	18	2
4	6	12	10	19	18
5	0	13	12	20	10
7	6	14	13	21	20
8	8	15	8	22	15
9	10	16	12	23	10
10	25	17	10	24	10
				26	0

From the results obtained on these four rabbits it is apparent that there was an accumulation of the toxic principle in the blood when the chemical at low dosages

was administered daily either by forced feeding or in the food. Host No. 38 was very favorable for retaining the chemical in the blood because, even at the low total dosage of 3 mg per kilogram, the toxic principle persisted for at least 16 days, which was longer than in other rabbits receiving single dosages ranging from 25 to 100 mg (see Table 2).

The low daily dosage of 0.1 mg per kilogram (host No. 69) caused some mortality, but this seems to be near the nontoxic level for lice receiving one blood meal.

There was no indication that the toxic principle in the blood increased in intensity after hosts Nos. 69 and 70 had been on treated food for about 10 days.

(4) *Effect on lice allowed several blood meals on rabbits that received single daily doses for several days.*—In natural infestations the lice might feed repeatedly or almost continuously on the host. Two experiments were therefore conducted in which lice were fed repeatedly on hosts that were consuming the chemical daily. One of these experiments involved forced feeding of the chemical, whereas in the other the host received the chemical in the food. In this type of experiment it was desirable to select favorable hosts so that the natural mortality of lice would not be too high.

Host No. 30 was chosen for the test with forced feeding. This animal was about 4 months old and weighed 2.13 kg. Previous feeding experiments had shown that this rabbit and a litter mate, host No. 31, which was used as a check, were both highly favorable, and equally so, for rearing lice. In fact, lice on these animals seemed to develop as well as can normally be expected when they are fed on man.

This host received daily dosages by capsule of 0.25 mg of 2-pivalyl-1,3-indandione in the form of a powder (1 per cent in pyrophyllite). Within 6 hours after the initial dose both nymphs and young adult lice were given their first blood meal. The same lice were then fed exclusively on this host for several days. They received two blood meals each day, at 8 AM and 4 PM. The number of doses to be administered to the host was dependent on the mortality of lice as the experiment progressed.

Two hundred adult lice (100 males and 100 females) and 400 newly hatched nymphs, which had previously had one blood meal on this host, were given blood meals on the treated host, and the same numbers of nymphs and adult lice from the same source were fed at the same time on the untreated check animal (host No. 31).

Daily records of mortality among the adult lice were obtained. Because of the difficulty in making the counts, no records were obtained on the nymphs during the first 2 days, but it was noted that mortality was negligible.

Table 3 shows the numbers of lice surviving on the treated and untreated rabbits. All the female lice that engorged on the treated rabbit died by the 4th day. By this time they had been offered five blood meals and the rabbit had received a total of 0.75 mg of the chemical per kilogram. Only 10 per cent mortality occurred during the same period among the female lice feeding on the untreated rabbit. The male lice on the treated rabbit survived longer than the female lice, whereas on untreated rabbits the females lived longer.

To determine whether the apparent difference in resistance between the sexes was due to differences in the amount of blood consumed, some precise data were obtained on the blood consumption of the two sexes. It was found that in relation to the body weight the females consumed about $2\frac{1}{2}$ times as much blood as did the males.

Table 3 shows also that the nymphs fed on the treated rabbit survived for a

longer time than did the adult females. Whether this difference can also be explained by differences in the amount of blood consumed in relation to the body weight is not known, but on general observations young nymphs appear to consume as much blood for their size as do adult females.

Host No. 69 was chosen for the test to determine the effect of daily doses of the chemical administered in the food. Rabbit food was treated with an acetone solution of 2-pivalyl-1,3-indandione so as to contain 5 parts per million of the chemical. Fifty grams of the food was given daily for 19 days, which provided a dosage of 0.1 mg per kilogram of body weight, since the animal weighed 2.5 kg.

Ten lots of 200 adult lice (100 males and 100 females) were permitted to feed exclusively on the host twice a day for 3 days (6 blood meals). The first lot were given the first blood meal on the 7th day after the animal had been placed on the

TABLE 3.—*Numbers of lice surviving daily when allowed two blood meals each day on a rabbit receiving 0.25 mg of 2-pivalyl-1,3-indandione per kilogram daily for 6 days, as compared with numbers surviving on an untreated host. 100 females, 100 males, and 200 nymphs on each host*

Days in test	Treated host (No. 30)			Untreated host (No. 31)		
	Adults		Nymphs	Adults		Nymphs
	Females	Males		Females	Males	
Tests made while rabbit was being fed treated food						
1	98	94	..	99	96	..
2	80	77	..	96	90	..
3	25	63	396	92	74	394
4	0	34	[1]	90	65	..
5	..	14	194	88	57	343
6	..	3	11	81	44	334
Tests made after rabbit was taken off the treated food						
7	..	1	5	71	33	323
8	..	0	3	63	25	306
9	..	..	0	52	15	294[2]
10	..	..	..	48	10	289
11	..	..	..	41	9	269
12	..	..	..	33	7	258
13	..	..	..	26	6	232
14	..	..	..	20	2	207

[1] No counts were made on this day, but at least 100 of the nymphs were paralyzed 6 hours after the 5 P.M. feeding.
[2] Nymphs began molting to adult stage on the 9th day, and all had reached the adult stage by the 12th day.

treated food. The second lot were started on the 12th day and the third lot on the 16th. On the 19th day the animal was taken off the treated food, but lice were fed for 3-day periods at intervals thereafter.

The survival data, presented in Table 4, show that a daily dosage of 0.1 mg per kilogram prevented development of the lice allowed repeated blood meals on the host. The lice started on the 7th day showed high survival during the first 24 hours, but within 2 days there were definite indications of toxic effect, and by the 3rd day most of the lice had died. The females again showed lower survival than did the males.

There was little difference in survival between the lice started on the 12th and the 16th days. It is apparent, however, that the toxic principle in the blood during both these periods was more pronounced than during the 7th to 9th days.

The persistence of the toxic agent in the blood after the animal was taken off the treated food was surprising. Although considerable variation is indicated, there was

still enough toxicant in the blood after 30 days (on 49th day after feeding was begun) to prevent normal development of the lice. From the 56th to 58th days the mortality was relatively slight, but it is believed that the drop in survival of females from 96 to 81 per cent between the 2nd and 3rd days is due in part to the chemical. There was no indication of toxic effect after the 63rd day, and it was assumed that the animal had eliminated the toxic principle from the blood.

2-Pivalyl-1,3-indandione is apparently absorbed by the blood of the host very quickly. The blood of one animal that received 25 mg per kilogram caused the death of all lice that fed 2 hours after the chemical was administered. The same was true of the lice feeding after 4 and 6 hours, but they showed paralysis more quickly.

The period of maximum concentration of the toxic agent in the blood of the host is not apparent from examination of data obtained against lice. However, results with mosquitoes, discussed in the section that follows, threw some light on this question.

TABLE 4.—*Survival of lice fed twice a day for 3 days on a rabbit (No. 69) receiving rations containing 0.1 mg of 2-pivalyl-1,3-indandione per kilogram each day for 19 days*

Days rabbit was fed before lice were given blood meal	Percentage of lice surviving					
	Females			Males		
	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Lice given blood meal while rabbit was being fed treated food						
7	99	44	19	95	68	43
12	32	7	2	74	45	27
16	65	13	0	80	25	12
Lice given blood meal after rabbit was taken off treated food						
21	70	26	5	82	59	27
28	59	11	2	74	32	8
35	87	30	8	71	42	32
42	94	62	20	90	68	44
49	87	34	16	90	67	46
56	98	96	81	98	96	90
63	100	99	94	99	94	90

Tests on Mosquitoes

Mosquitoes were fed on the same animals which received orally 100- and 25-mg dosages of 2-pivalyl-1,3-indandione and already discussed in connection with tests on lice. Two animals received the chemical in crystalline form and two in oil. The results are shown in Table 5.

The chemical was not highly effective against mosquitoes, although some toxic action was produced at both dosages. These data indicate that the toxic agent in the blood reaches a maximum concentration some time within 24 hours after the chemical is administered to the host, and apparently it is usually higher after 6 hours than after 24.

Tests on Ticks

In tests to determine the effect of 2-pivalyl-1,3-indandione on ticks, two animals receiving single oral doses were used.

One animal received 25 mg per kilogram and was infested on the same day with 25 nymphs and approximately 75 larvae of *Amblyomma americanum*. An untreated animal was infested at the same time. Of 23 nymphs that attached to the treated animal within 24 hours, only 1 engorged. Engorgement was completed on the 5th

day, but some were alive as long as 4 days after attachment. Of 24 nymphs that attached on the untreated rabbit, at least 12 engorged, all of which completed engorgement by the end of the 3rd day. About 25 larvae attached on each animal. None engorged on the treated animal, whereas most of them engorged on the untreated host.

TABLE 5.—*Mortality after 24 hours of mosquitoes allowed blood meals on rabbits on indicated days after administration of 2-pivalyl-1,3-indandione*

Dosage (mg/kg)	Form	Host No.	Per cent mortality of mosquitoes fed after—				
			½ day	1 day	2 days	3 days	4 days
100	Crystalline	4	100	40	10	13	0
	In oil	17	95	24	10	14	0
25	Crystalline	1A	9	50	14	..	..
	In oil	23	80	18	8	..	..

The second animal received 5 mg of the chemical per kilogram of body weight. Little or no effect was noted on the tick larvae and nymphs.

TESTS WITH OTHER INDANDIONE DERIVATIVES

The results against lice with indandione compounds other than 2-pivalyl-1,3-indandione are shown in Table 6. All of the compounds except the potassium salt of N,N-diethyl-2-butyrylamino-1,3-indandione showed some action on lice. The sodium salt of 2-isovaleryl-1,3-indandione proved more toxic than the parent compound, and in the host that received 100 mg per kilogram (No. 72) the toxic agent persisted in the blood for a long time. None of the other derivatives equaled the parent compound in effectiveness.

TABLE 6.—*Mortality of lice 24 hours after being allowed single blood meal on rabbits receiving oral doses of various indandione compounds*

1,3-Indandione derivative	Dosage, mg/kg	Host No.	Per cent mortality of lice fed on indicated days after administration of chemical to host						
			½ day	1 day	2 days	3 days	4 days	5 days	6 days
2-Caproyl	300	94	98	[1]	..	..	..	..	..
	25	97	98	66	3	..	..	..	..
2-Cyclopropylcarbonyl, potassium salt	300	92	70	100	100	58	48	..	0
	25		28	98	34	..	0	..	..
N,N-diethyl-2,butyrylamino, potassium salt	300	93	2	3	5	..	..	..	..
2-Enanthyl	300	95	98	[1]	..	..	..	..	..
	25	98	100	8	..	..	..	..	..
2-Isovaleryl	300	16	90	30	65[1]	..	..	..	..
2-Isovaleryl, sodium salt	300	22A	100	100[1]	..	..	..	..	..
	100	72	100	98	98	92	97	35	72[2]
	5	62	92	13	10	..	..	..	..
2-Pivalyl, sodium salt	2.5	71	93	100	80	64	30	0	..
	2.5[3]	36	86	94	80	45	60	20	..
	1[3]	87	0	2	5	2	..	..	..

[1] Rabbit died.
[2] Additional mortality records as follows: 8th day—65, 9th—69, 10th—33, 11th—62, 12th—15, 13th—26, 14th—63, 15th—50, 17th—100, 18th—48, 19th—no record, 20th—0.
[3] Chemical in salt solution applied subcutaneously.

SOME INCIDENTAL OBSERVATIONS ON THE EFFECTS OF INDANDIONE COMPOUNDS

When indandione compounds were administered to rabbits in large doses, the lice subsequently given blood meals were killed very quickly. At 300 and 100 mg per kilogram of body weight they were immobilized by the time they completed feeding, usually within 10 to 20 minutes. The toxic action apparently took place

without disturbance to the lice, for they died while in the feeding position. However, while feeding, the lice developed a marked reddish appearance over the entire body. It is believed that the blood permeates the gut wall and enters the hemolymph of the insect. This reddening developed more slowly and was less pronounced at the lower dosage levels, or when the concentration of the toxic agent in the rabbit's blood reached a low level. The time required to paralyze the lice was also greater as the dosage was decreased, or when the higher dosages began to become ineffective. Lice fed on animals that received 25 mg of the chemical per kilogram generally became paralyzed in 1 to 2 hours, whereas at a dosage of 5 mg per kilogram the paralysis time was from $1\frac{1}{2}$ to 7 hours. Lice not affected within 12 hours usually did not die within 24 hours. Once paralyzed, however, they apparently did not recover.

TOXICOLOGY OF INDANDIONE COMPOUNDS

At the highest dosage, 300 mg per kilogram, most of the indandione compounds caused the death of the host (see Table 6). The toxicological effects of the chemicals were not studied as a major aspect of the investigations, even though it is recognized that this may eventually be an important consideration. Weight records were recorded at irregular intervals, however, for most of the animals receiving 5 mg or less of 2-pivalyl-1,3-indandione per kilogram of body weight, and these animals continued to gain in weight. The animals receiving 0.1 and 0.25 mg per kilogram daily in the feed for 19 days also showed slight weight gains during and after the feeding period.

Kabat, Stohlman, and Smith (1944) have made a rather thorough pharmacological investigation of 2-pivalyl-1,3-indandione and certain other indandione derivatives. They report that this chemical caused the death of four out of five rats when administered orally, in olive oil, at a dosage of 150 mg per kilogram, and that dosages of 200 and 400 mg per kilogram killed all rats (three at each dosage). Of four rats receiving 100 mg per kilogram none were killed. Results with rabbits were somewhat similar. The toxicology of 2-isovaleryl-1,3-indandione to rats and rabbits is reported to be of the same order as is 2-pivalyl-1,3-indandione.

Of particular interest, as reported by these authors, is the effect of the indandione compounds on the blood of the host. They found that death of the animals was due principally to hemorrhage, and that hypoprothrombinemia was produced at dosages as low as 1 mg per kilogram.

DISCUSSION

The studies conducted thus far on internal therapy for external bloodsucking arthropod parasites should stimulate interest in more intensive studies in this field. In some respects the exploratory studies herein reported were not encouraging, since most of the highly effective insecticides known at the present time proved ineffective. On the other hand, the results with 2-pivalyl-1,3-indandione against lice exceeded by far the writers' expectations. The results with gamma-benzene hexachloride against mosquitoes and ear mites and with 2-pivalyl-1,3-indandione against ticks, although considerably less spectacular, further demonstrate that external bloodsucking parasites can be destroyed by the administration of relatively low dosages of a chemical to the host.

Of the materials tested thus far there is no close relationship between their value

as contact insecticides and their effectiveness as internal medicants. G. W. Eddy and R. C. Bushland, of the Orlando, Fla., laboratory, found that gamma-benzene hexachloride, chlordane, chlorinated camphene, 2-pivalyl-1,3-indandione, and DDT were all highly effective as contact insecticides for lice (unpublished manuscript). However, 2-pivalyl-1,3-indandione was the only one that proved highly toxic to lice which engorged on rabbits receiving these chemicals. As a therapeutic agent the indandione compound proved approximately 300 times as toxic as the most effective of the other four. On the other hand, dicumarol, which possesses no value as a contact insecticide against lice, was far more effective when administered to the host than were the highly effective contact insecticides mentioned, excluding 2-pivalyl-1,3-indandione. Studies to explain the difference in activity of the various materials as chemotherapeutic agents would be desirable.

In view of the promising results with 2-pivalyl-1,3-indandione as a chemotherapeutic agent for body lice and ticks, and with benzene hexachloride for mosquitoes and mites, the next logical step is to determine the value of these and various other chemicals when administered to animals harboring natural infestations of lice, mites, fleas, flies, and other parasites. Should 2-pivalyl-1,3-indandione prove as effective against other external parasites as it is against body lice, it might be incorporated in feed, salt, mineral mixtures, or water consumed by the host. Although toxicity is a major consideration, tests should be conducted under close and careful supervision with 2-pivalyl-1,3-indandione administered to various animals harboring different bloodsucking parasites, to determine the effect on the parasite and on the host.

Aside from the possible development of indandione compounds and other chemicals as chemotherapeutic agents for controlling animal parasites, the information gained may prove useful in other ways. Studies employing body lice or other insects as indicators, might be made to explore methods of formulating and administering chemicals or drugs to obtain maximum effectiveness for the treatment of parasites and diseases of various kinds.

SUMMARY AND CONCLUSIONS

Certain insecticides and drugs were administered internally to rabbits to determine their value as chemotherapeutic agents against body lice (*Pediculus humanus corporis* Deg.) and yellow-fever mosquitoes (*Aedes aegypti* (L.)) that were subsequently allowed to take blood meals on the treated hosts. Several chemicals were evaluated also against the ear mite (*Psoroptes equi* var. *cuniculi* (Delafond)) and against the lone star tick (*Amblyomma americanum* (L.)).

In the initial tests against lice and mosquitoes the chemicals were usually administered orally at a dosage of 300 mg per kilogram of body weight. A total of 33 chemicals were tested against lice and 31 against mosquitoes. Further tests at lower dosages were made with the materials that caused complete kill. Observations were made on the effect of 9 chemicals on mites and of 2 materials on ticks.

The major findings and observations in connection with these studies are summarized below:

(1) The following chemicals at dosages of 300 mg per kilogram or less possessed definite toxic action as chemotherapeutic agents when lice were allowed one blood meal on the treated host: gamma-benzene hexachloride; 2-caproyl-1,3-indandione; chlordane; potassium salt of 2-cyclopropyl-carbonyl-1,3-indandione; chlorinated

camphene; 2-enanthyl-1,3-indandione; 2-isovaleryl-1,3-indandione; sodium salt of 2-isovaleryl-1,3-indandione; 2-pivalyl-1,3-indandione; sodium salt of 2-pivalyl-1,3-indandione; dicumarol; powdered sabadilla seed.

(2) When mosquitoes were allowed to engorge on the treated rabbits, the following chemicals at dosages of 300 mg per kilogram or less showed some action: gamma-benzene hexachloride; chlordane; potassium salt of 2-cyclopropylcarbonyl-1,3-indandione; 2-isovaleryl-1,3-indandione; sodium salt of 2-isovaleryl-1,3-indandione; 2-pivalyl-1,3-indandione; chloromethyl *p*-chlorophenyl sulfone; and dicumarol.

(3) Gamma-benzene hexachloride was the only material that caused complete mortality of mosquitoes at the initial dosage of 300 mg per kilogram. In subsequent tests with this material administered in corn oil or in crystalline form at 100 and 25 mg per kilogram, complete kill of mosquitoes was obtained for several days although the chemical proved quite toxic to the host. A dosage of 5 mg per kilogram caused 93 per cent mortality of mosquitoes that were given a blood meal 24 hours after the chemical was administered to the host.

(4) Several of the indandione compounds caused complete mortality of lice when administered to the host at the rate of 300 mg per kilogram, although most of the rabbits died within a few days to a week. The most effective chemical, 2-pivalyl-1,3-indandione, was tested extensively at various dosages and by different methods of administration. On the basis of these tests, the following tentative conclusions were drawn:

(a) Oral dosage of 5 to 100 mg per kilogram caused complete mortality of lice allowed a single blood meal and the duration of complete kill varied from 3 to 8 days, with partial kill for several days thereafter. There was little relation between the dosage and the duration of the toxic effect.

(b) An oral dosage of 2.5 mg per kilogram caused complete or nearly complete kill of lice allowed one blood meal, whereas 1 mg per kilogram caused mortalities ranging from 18 to 30 per cent in three rabbits.

(c) Subcutaneous, intramuscular, and intravenous injections at the rate of 2.5 mg per kilogram produced results comparable with those obtained with oral administrations at the same dosage. Some kill of lice was obtained also when the chemical was administered (in solution) by inunction through the unbroken skin, although results were erratic.

(d) There was a definite accumulation of the toxic agent in the blood of the host when the chemical was administered orally each day for several days.

(e) There was likewise a definite cumulative effect of the toxic principle in lice allowed repeated blood meals on rabbits receiving daily doses of the chemical. A rabbit which received daily for 19 days 0.1 mg per kilogram of the chemical in food caused complete to nearly complete mortality of female lice in different lots allowed six blood meals each after 1 week and during the rest of the time the animal was on treated food. The toxic agent persisted in the blood of the host for several weeks after the rabbit was taken off the treated food.

(f) Female lice showed a higher mortality when they engorged on treated hosts than did the males, but this is explained by the fact that, on the basis of body weight, the females consume approximately $2\frac{1}{2}$ times as much blood as do the males.

(g) Rabbits receiving 5 mg or less of the chemicals per kilogram in single doses and those receiving 0.1 and 0.25 mg per kilogram per day in food for 19 days

showed no apparent injury due to the chemical, and they continued to gain weight.

(5) Ear mites were killed by single doses of gamma-benzene hexachloride given to hosts at the rate of 100 to 300 mg per kilogram. Dosages of 50 mg per kilogram caused only partial kill of the mites.

(6) Larvae and nymphs of the lone star tick were not affected when allowed to engorge on a rabbit receiving an oral dosage of 25 mg of gamma-benzene hexachloride per kilogram.

(7) 2-Pivalyl-1,3-indandione given to a rabbit at the rate of 25 mg per kilogram prevented engorgement of all larvae and most of the nymphs of this tick, but 5 mg per kilogram proved ineffective.

(8) There is little relation between the value of some of the chemicals as contact insecticides against lice and mosquitoes and their value as chemotherapeutic agents. As contact insecticides against lice DDT, benzene hexachloride, chlordane, and chlorinated camphene are about as effective as 2-pivalyl-1,3-indandione, but when administered to rabbits as chemotherapeutic agents, the last material was 300 or more times as toxic.

(9) Chemotherapeutic agents for bloodsucking insects apparently show high specificity. 2-Pivalyl-1,3-indandione proved highly effective against lice and relatively ineffective against mosquitoes, whereas the gamma-benzene hexachloride was much more effective against mosquitoes than against lice.

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RESEARCH NOTES

A SUCCESSFUL METHOD FOR SHIPPING LARVAL TROMBICULIDS (CHIGGERS)

The method presented here was developed in connection with a project supported by a grant from the division of Research Grants and Fellowships, U. S. Public Health Service and has been used successfully to ship living chiggers. The special vials are prepared in the laboratory, together with blank information labels, and placed in $4\frac{3}{8}'' \times 1\frac{3}{4}''$ paraffin-sprayed, screw-top mailing cartons, from 1 to 6 special vials being packed in excelsior or cotton batting in each. A number of these, in a box of convenient size, are shipped to the collector in the field. He places chiggers from each host in a separate vial, attaches a completed information label with a rubber band, and repacks the vials into the pre-addressed mailing cartons. The cartons are then mailed with as little delay as possible.

The special vials are prepared from clean 25×15-mm shell vials stoppered with No. 4 corks. New, uncontaminated corks are selected with great care; only those with the smaller end smooth and absolutely free of pits and imperfections are used, to avoid offering the mites hiding places. The vials are completely lined with a mixture of plaster of Paris 90 per cent by weight and activated charcoal 10 per cent by weight. The two powders are weighed separately, placed together in a jar sufficiently large to permit liberal agitation, and thoroughly mixed by shaking. Lumpy powders are to be avoided. Materials are kept in clean glassware and assembled over clean paper towels. By means of a small spatula the vials are 2/3 filled with the dry mixture. About 16 drops of distilled water are added and mixed with the powders by use of the spatula. By rotating the vial and manipulating the spatula, the sides and bottom of the vial are easily and completely covered. The vial is stoppered immediately. Finished lined vials ordinarily are not autoclaved. Any corks or vials being made up after having once been used are autoclaved at 15 pounds per square inch for 5 minutes.

These specially lined vials maintain a satisfactory atmosphere for the chiggers as far as relative humidity is concerned. Fungus also is eliminated largely, if the collector will avoid placing organic debris in the vials. Ears and other parts of host animals with attached chiggers must not be placed in the vials. When vials are received in the laboratory, they should be examined carefully for chiggers with a dissecting microscope, the cork being given a full share of attention.

This method has been used successfully in over 70 shipments of chiggers. In about 8 additional cases, pieces of ear were included and only a few chiggers were recovered. In 15 shipments in plain glass tubes, all specimens arrived dead.

Further, those specimens which are to be reared are left in the special vials. If a drop of distilled water is added occasionally, the vials make good containers in which engorged chiggers will transform into nymphs.

The authors wish to express their thanks to Neil B. Richmond, Woodrow Goodpaster, Clay Gifford and William Grimm of the Pennsylvania Game Commission who cooperated by collecting and shipping the specimens to them.—Charles E. Farrell and G. W. Wharton, *Departmtnt of Zoology, Duke University, Durham, North Carolina.*

SPIROMETRA FROM TEXAS CATS

Recently the writer had the opportunity to examine the viscera from fourteen stray cats acquired locally by the Department of Biology of the Rice Institute for experiments in physiology. Two of these cats were found to be harboring tapeworms of the genus *Spirometra* Mueller, 1937. One cat was infected with two worms; the second yielded a single immature specimen.

Two species of this genus have been reported from North America; these are *S. mansonoides* Mueller and a second unnamed species recovered by Mueller (1938, Liv. Jub. Prof. Trav.) from cats infected in the laboratory with spargana from Florida *Natrix* sp. Mueller regarded the domestic cat as an abnormal host.

The worms from Houston cats are similar in measurement to the unnamed species of Mueller. The mature specimens measure 12.8 and 13.0 cm in length and a maximum of 1.5 cm in width. The segments number 260 and 286 per respective worm. The scolex is about 1.0 mm long and very thin and delicate. The neck is about 1.0 cm long. The anatomy of the segments seems identical to that of Mueller's species, except that the mature segments are not noticeably longer than broad, but are approximately square; this is probably due to differences in preparation.

S. mansonoides has not been reported from Texas. Dr. Asa C. Chandler kindly loaned the writer six slides containing portions of a tapeworm strobila. This material is labeled "Bobcat, South Texas" and is presumably from *Lynx rufus*. Examination of the material and direct comparison with specimens of *S. mansonoides* Mueller, also loaned by Dr. Chandler, revealed that the material from Texas bobcat is specifically identical with *S. mansonoides*.

It is interesting to note that the first case of human sparganosis reported from the United States was reported from this city by Moore (1915, Am. J. Trop. Dis. Prev. Med.).—CLARK P. READ, *Department of Biology, The Rice Institute, Houston, Texas.*

DIPHYLLOBOTHRIUM INFECTION IN FLORIDA

Diphyllobothrium latum infection was reported in three negro children and a dog from Jefferson County, Florida, by Summers and Weinstein (1943, Am. J. Trop. Med. **23**: 363–367). The children were treated and a total of 33 worms recovered.

During 1946 the parasitology division of the Florida State Board of Health examined 640 fecal specimens from individuals residing in Jefferson County without finding any *D. latum* eggs. It seemed advisable, however, to make a more intensive study of this negro community from which the infection had previously been reported.

A survey of the area was made in March, 1947. Specimens were examined from 137 individuals, mostly children, and from six dogs. One of the boys who had previously harbored the parasite and had received treatment still showed *D. latum* eggs in the stool. One dog belonging to a family of "kin folk" was also found infected.

In April, 1947 the members of the two families, 13 in all, and four of their dogs were reexamined. No additional cases were found. The infected dog was purchased, shot and examined. *D. latum* eggs were present in the fecal material but no worms were found, although the gastrointestinal tract was carefully examined from the cardiac portion of the stomach through the anus. In response to an inquiry, Dr. E. C. Faust of Tulane University (personal communication) stated "The infection spontaneously disappears in the dog rather soon after the worm matures."

Early in June, 1947 the infected child was treated with oleoresin of aspidum under the supervision of Dr. B. L. Arms, Director of the Jefferson County Health Unit. Seventeen *Diphyllobothrium latum* worms were recovered, each complete with head. The individual worms varied in length from 10 to 41 inches. The total worm burden was 31 feet 8 inches. A final stool specimen from this boy was examined on August 18, 1947. No *D. latum* eggs were found either on direct examination or on concentration by sedimentation.

There is no evidence at present of a residual *D. latum* infection in this area. The fact that such an infection did occur in this locality, reemphasizes the need of a constant alertness to recognize the unusual when it is presented.—MARION HOOD, *Charity Hospital of Louisiana at New Orleans; formerly Senior Parasitologist, Florida State Board of Health.*

Mailing dates of Volume 33, Journal of Parasitology

February issue	March 6, 1947
April issue	May 9, 1947
June issue	July 1, 1947
August issue	August 29, 1947
October issue	November 20, 1947
December issue	
Section 1	December 12, 1947
Section 2	February 23, 1948

FOURTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

The Fourth International Congresses on Tropical Medicine and Malaria, which will be held in Washington, D. C. from May 10 to 18, 1948, will be of very great interest to American parasitologists. The Congresses will be sponsored by the Government of the United States through its Department of State, which has invited more than 60 governments to send official delegates. Already the attendance of a large number of foreign parasitologists is assured.

Two important evening meetings of special interest to parasitologists will be held, one commemorating the establishment by Walter Reed of the mosquito transmission of yellow fever and celebrating his admission to the Hall of Fame, and the other commemorating the 50th anniversary of the discovery by Ronald Ross of the method of transmission of malaria. There will be an extensive series of motion pictures, many on parasitic diseases and their transmission, shown each day from Monday, May 10 to Saturday, May 15, and numerous scientific and commercial exhibits.

A large proportion of the regular scientific sessions of the Congresses will be devoted to parasitology. The meetings on malaria are especially extensive, with six sessions covering all phases of the subject. Four sessions on medical and veterinary entomology cover the various groups of arthropods and the recent work on insecticides. The two sessions on protozoology deal with amebiasis and blood and tissue flagellates, and the two sessions on helminthic diseases emphasize filariasis, schistosomiasis, and hookworm diseases. Also, there are programs on arthropod-borne virus and rickettsial diseases, and papers on trypanosomiasis and schistosomiasis in the section on tropical veterinary medicine.

In addition to the scientific sessions, there will be an official reception, hospitality sessions, a day's visit to the Department of Agriculture Experimental Farm at Beltsville, a boat trip to Mount Vernon, a garden party at the Pan American Union, a general dinner for all members of the Congresses, and visits to the National Institute of Health, Naval Medical Research Institute, Army Medical Center, and other scientific institutions in and near Washington. There will also be a number of special events for the entertainment of visiting ladies and opportunities for them to visit points of interest in Washington.

A number of members of the American Society of Parasitologists are on the program as conveners, regular speakers, and commentators. It is hoped that the attendance from the Society will be large, since it will be a fine opportunity to meet with foreign workers in our field.

Dr. Wilbur A. Sawyer is the Executive Secretary of the Congresses. Requests for advance registration blanks and information should be addressed to him at the Division on International Conferences, Department of State, Washington 25, D. C. Members of the American Society of Parasitologists or others interested in tropical medicine may become members of the Congresses by paying the registration fee of ten dollars which includes a copy of the Proceedings. Students and members of the families of members of the Congresses can become associate members by paying a registration fee of five dollars, which does not include the Proceedings. Early registration is recommended to assure good hotel reservations. The registration fees and requests for hotel reservations should be sent to Mr. Ernest F. Gamache, Treasurer, 270 Madison Avenue, New York 16, N. Y.

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